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FRONT COVER

Contract No.: DAMD17-88-C-8011

Title: Synthesis and Biological Evaluation of Brain-Specific Anti-RNA Viral Agents

Principal Investigator: Marcus E. Brewster, Ph.D.

PI Address: Pharmatec, Inc.
P.O. Box 730
County Road 2054
Alachua, FL 32615

Report Date: 30 June 1989

Type of Report: Midterm

Proposal for: U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, MD 21701-5012

Distribution Statement:

Approved for public release; Distribution Unlimited

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Pharmatec, Inc. / 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): P.O. Box 730, County Road 2054, Alachua, FL 32615	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command / 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-88-C-8011
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO.	WORK UNIT ACCESSION NO.
61102A	61102BS12	AD	114

11. TITLE (Include Security Classification): Synthesis and Biological Evaluation of Brain Specific Anti-RNA Viral Agents

12. PERSONAL AUTHOR(S): Marcus E. Brewster, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Midterm	FROM 11/30/87 TO 5/31/89	1989 June 30	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Ribavirin, brain, targeted delivery, RNA viruses, dihydropyridines, Punta Toro virus, RA I
07	03		
06	01		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Ribavirin is a potentially useful drug for treating encephalitic disease but is excluded from the brain because of its poor membrane permeability. This contract has attempted to develop brain-targeted delivery forms of ribavirin in an attempt to increase its efficacy towards central maladies. Ten such delivery systems have been prepared. These include several in which a brain-selective 1,4-dihydrotrigonellinate was attached to the 5'-position, one in which the 2'-position was modified and several others. After preparation, analytical systems were developed to separate, detect and quantitate these materials both in vivo and in vitro. The stability of selected compounds was then studied in various biological matrices. Further in vivo testing included tissue distribution studies and antiviral activity studies performed in a murine viral encephalitic model.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code): 301/663-7325	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

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Thomas E. Bennett 6/30/89

PI - Signature Date

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INTRODUCTION

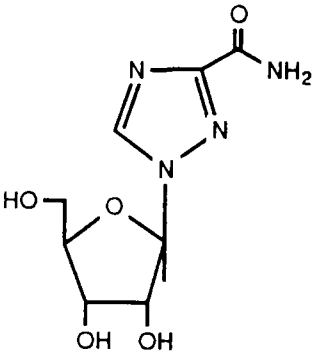
The inability to adequately treat viral encephalitic disease has made this malady pernicious and often fatal[1,2]. The poor therapeutic accessibility of these infections can be traced to three major facets including the viral life cycle, the lack of efficacious pharmacologically-active agents and finally the inability to deliver those agents which are available to the central nervous system (CNS) for sustained periods and in significant amounts.

Viruses are submicroscopic pathogens which depend on the cellular nucleic acid and protein synthesizing mechanisms of its host for propagation[1,2]. In general, viruses invade cells by first interacting at a recognizable surface protein, penetrating the cell membrane and subsequently releasing itself from a protective polypeptide coat to eject the core of the virus. The heart of these pathogens is genetic material either DNA or RNA and the type of nucleic acid give rise to the system of nomenclature for these entities. The viral DNA or RNA can then interact with cellular components to produce daughter genetic material as well as various structural or enzymatic proteins. After assembly and release, the viral progeny may infect other cells yielding disease or ultimately death.

DNA viruses are subdivided into five families and include the pathogens responsible for labial and genital herpes, chicken pox, shingles and mononucleosis. RNA viruses are present in more numerous forms and are subdivided into ten families. These viruses are unusual in that they reverse the usual DNA → RNA → protein sequence which occurs in higher life forms. RNA viruses are unusually dangerous for several reasons including their lethality and the lack of effective treatments. RNA viral diseases include hemorrhagic fevers of various descriptions, Dengue fever, Lassa fever and numerous encephalitic maladies including Japanese B encephalitis[2,3].

Chemotherapeutically, very few antiviral agents have been developed that have high in vitro activity against these virus. One notable advance in the field was the advent of ribavirin or 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide which was synthesized in 1972[4,5]. Ribavirin has a broad range of activity against both DNA and RNA viruses[6-9]. This riboside, which contains an unnatural triazole base, significantly suppresses the infectivity and cytopathicity of several viral pathogens by mechanism

which are as of yet unclear. Several interactions have been suggested including inhibition of viral RNA polymerase[10,11], the inhibition of inosine monophosphate dehydrogenase by ribavirin anabolites[9,12] and interference of mRNA cap formation by the 5'-triphosphate of ribavirin[12].



Ribavirin

These laboratory studies have been successfully translated to a clinically effective product. Ribavirin is active against several influenza viruses and respiratory syncytial virus and as such is used in an aerosol form to treat these diseases[13,14]. Ribavirin is also used in the treatment of Lassa fever which rages in epidemic proportions in Sierra Leone[15]. This regimen is significantly more effective than placebo in increasing survival.

Unfortunately, while peripheral viral infections can be successfully treated with ribavirin and other riboside derivatives, encephalitis is immune to the action of these drugs[16,17]. The ability of antiviral drugs, which are highly potent in vitro, to exert activity in the CNS is attributable to their exclusion from the brain. The basis of this impermeability is the blood-brain barrier (BBB) which effectively separates the systemic circulation from the brain parenchyma. The capillaries which make up the cerebral microvasculature differ in three important morphological and several histochemical respects from their peripheral counterparts[18,19]. Structurally, CNS microvessel are unique in the way in which the component endothelial cells are joined together. In the brain, gaps between adjacent endothelial cells are significantly more narrow than in the systemic circumstance thus preventing the bulk transport of materials between cells and forcing compounds to diffuse directly through the cell membranes. As these barriers are

lipoidal in nature, the BBB restricts the entry of materials which do not have high affinity for the phospholipid matrix and consequently hydrophilic compounds are excluded. In addition to the morphological adaptations, brain capillaries lack fenestra and have a vesicular transport system which is less active than that found in the periphery. Cerebral capillaries are also characterized by their high content of various lytic enzymes which prevent uptake of blood-borne neurotransmitters and other substances. Superimposed on the relatively impermeable system are several selective and saturable carrier system to allow for the bidirectional equilibrium of various nutrients and metabolic wastes[20]. As these specialized carriers usually are not important in drug uptake, molecules must be intrinsically lipophilic if they are to gain access to the CNS. This is the restriction which renders ribavirin which has a log P of only -2.06 ineffective in treating viral disease of the brain[11]. Experimentally this is borne out by the inability of ribavirin to increase the life span or reduce viral titres in laboratory animals inoculated intracerebrally with various "susceptible" viruses.

Thus, as indicated in the premise, three major problems are encountered in treating brain viral disease. Assuming compounds like ribavirin would be effective if transported adequately to the CNS, efforts should be directed toward improving brain delivery of this riboside. One method for approaching this problem is transient chemical derivitization of ribavirin via the prodrug method[21,22]. A prodrug is defined as a pharmacologically inert chemical derivative of an active compound which converts to the active species in vivo. The chemical manipulations are designed to transiently improve some deficient physicochemical property such as membrane permeability. In the case of ribavirin, esterification of the sugar hydroxy groups can lead to an increase in the lipophilicity of the conjugate. This technique has in some cases marginally improved the action of ribavirin against cerebrally implanted viruses. The triacetate of ribavirin was shown to increase the mean survival time and number of survivors when give i.p. to animals inoculated intracerebrally with Colorado tick fever virus[23] and Dengue virus[24]. Similarly, the tributyrate of ribavirin when administered subcutaneously was shown to increase the mean time to death in animals intracerebrally infected with Junin virus[25].

These results are encouraging in that increasing the concentration of ribavirin in the CNS by temporarily increasing its lipophilicity may lead to an improvement in the

action of the compound. The lipophilic esters chosen appear to be inactive in and of themselves but hydrolyze to yield the active antiviral agent. These prodrugs are not, however, optimized in terms of their pharmacokinetic and tissue distribution profile. While it is true that by increasing the lipophilicity of ribavirin, the drug will more easily pass the BBB and enter the CNS, the increased lipophilicity will increase the distribution of the conjugate in general leading to a greater tissue burden in non-target loci. This is important to consider when potentially cytotoxic materials are concerned. The other major drawback of simple lipophilic prodrugs is that while influx to the CNS is increased, efflux is likewise enhanced with the result being poor brain retention and a therapeutically short biological half-life. These two objections to simple prodrug, that is increase tissue burden with little tissue selectivity and poor CNS retention prompted the application of a novel drug targeting system. The method selected is the Chemical Delivery System (CDS)[26-28]. The CDS relies on the facile interconversion of a lipophilic dihydronicotinate and a hydrophilic nicotinate salt to achieve tissue selectivity and biomimics the conversion between NAD^+ and NADH. This approach requires that a molecular carrier be attached to the drug of interest. While various carriers can be used, derivatives of nicotinic acid have proved to be the most successful to date. Upon esterification with nicotinic acid or a nicotinic acid derivative, quaternization and reduction, a 1,4-dihydrotrigonellinate (or CDS) derivative is obtained. This lipophile can, after systemic administration, pass the BBB and enter the CNS as well as other tissue compartments. The metabolically unstable dihydropyridine then oxidizes to give the corresponding pyridinium salt. This ideally inactive species is rapidly lost from the periphery due to its hydrophilic nature but is retained in the CNS because of its polarity and size and, therefore, its inability to back diffuse through the BBB. With time, hydrolysis can free the drug from its inactive, depoted conjugate so that the liberated agent can exert its pharmacological effects. If the rate of degradation of the drug-oxidized carrier conjugate is slow, then a sustained release of the drug may be achieved. The advantages of this scheme include the low levels of the parent drug presented to the periphery which should reduce systemic dose-related toxicities. In addition, since the majority of the drug is present in the CNS as an inactive conjugate, central toxicity should be attenuated. This system has been successfully applied to a number of drugs

and neurotransmitters including antibiotics, antiviral agents, anticancer agents, steroids and many others[29-36].

In applying this approach to ribavirin, the chemical complexity of the molecule requires careful planning in terms of synthetic manipulations. Ribavirin is a riboside and thus contains three hydroxy groups, one primary (5') and two secondary (2' and 3'), which may provide handles for attaching either the brain-targeting dihydrotrigonellinate group or lipophilicity modifying esters or other derivatives. In addition, the triazole-carboxamide base can provide synthetic points of attachment via the amide group. As each of the hydroxylic and amide groups will provide derivatives with differing reactivities, the rates of oxidation and hydrolysis can be slowly optimized via selective synthetic manipulations. Subsequent to chemical alterations, preliminary toxicity screens were conducted and acceptable compounds examined analytically in both in vitro and in vivo paradigms. Finally, candidates were submitted and tested for biological activity in an encephalitis viral model utilizing the Balliet strain of Punta Toro virus[37].

BODY

RESULTS AND DISCUSSION

Chemistry

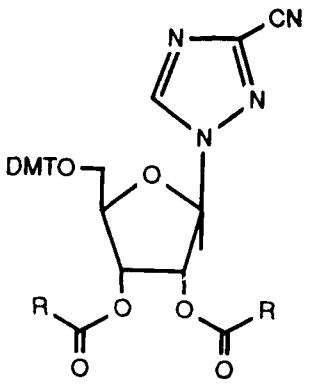
Several synthetic approaches have been examined and include attachment of a 1-methyl-1,4-dihydronicotinated moiety to the 5'-position of ribavirin followed by manipulation of the 2' and 3'-loci (5'-based CDS), attachment of the targeting moiety to the 3'-position of ribavirin followed by manipulation of the 2' and 5'-positions (3'-based CDS) and attachment of the dihydrotrigonellinate to the 2' position followed by manipulation of the 3' and 5'-hydroxy groups (2'-based CDS). In addition, attachment of the 1,4-dihydrotrigonellinate to more than one site was considered and derivitization of the carboxamide group attempted.

5'-Based Chemical Delivery Systems

To this point, the majority of delivery systems constructed are based on 5'-

attachment of the dihydronicotinate. In developing the methodologies for this synthetic work, a model system was first prepared. In this synthetic route (Scheme I), the 2',3'-hydroxy groups of ribavirin (1, AVS 0001) was protected as the 2',3'-0-isopropylidene (2, AVS 5221). The protected riboside was then reacted with trigonelline anhydride (3) to produce the 5'-trigonellinate iodide of the protected sugar (4). Reduction of this quaternary salt in aqueous basic sodium dithionite yielded the 5'-(1,4-dihydrotrigonellinate) of the ribavirin acetonide (5, AVS 5505). In the above synthesis, trigonelline anhydride was used to avoid possible complications arising from alkylation which may occur on the triazole base. The use of trigonelline anhydride, while effective, was cumbersome in that the trigonelline side product was difficult to remove, a circumstance which compromised the reaction yield. In circumventing this limitation, the synthetic route was re-examined by reacting the ribavirin acetonide with nicotinic anhydride giving rise to the 5'-nicotinate (6). This ester was subsequently quaternized to give (4a) and reduced to give (5). In the reaction conditions used for the quaternization, methylation of the triazole base was not observed.

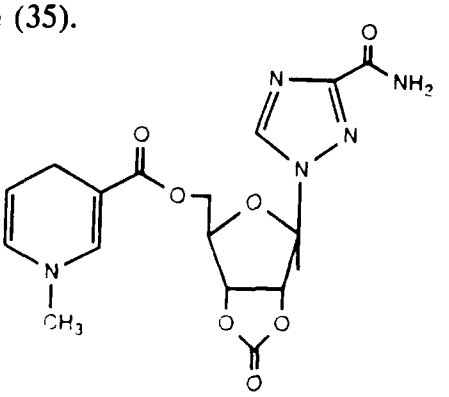
A series of 5'-CDSs were then prepared by a general process summarized in Scheme II. In the compound prepared, a 1,4-dihydrotrigonellinate moiety was attached to the 5'-position and bis acylation effected at the 2' and 3'-positions. Ribavirin was first protected using 4,4'-dimethoxytrityl chloride to give the 5'-(4,4'-dimethoxytrityl)ether derivative (7). The 2' and 3' hydroxy groups were then esterified with a variety of acid anhydrides including pivaloyl, benzoyl, isobutyryl and acetyl to give rise to the corresponding 5'-1,4-dihydrotrigonellinate-2',3'-diesters ((8), (9), (10) and (11), respectively). These compounds were then detritylate ! via the action of 80% acetic acid to yield compounds (12), (13), (14) and (15), respectively. The nicotinate carrier was then introduced. Use of nicotinoyl chloride hydrochloride as the acylating agent lead invariably to dehydration of the carboxamide of the base yielding the nitrile.



Use of nicotinic anhydride avoided this problem and gave the corresponding 5'-nicotinates in good yields ((16), (17), (18) and (19), respectively). Quaternization of these derivatives with methyl iodide gave the trigonellinates (20), (21), (22) and (23) and reduction of these salts with aqueous basic sodium dithionite gave the CDS's ((24, AVS 5056), (25, AVS 5057), (26, AVS 5054) and (27, AVS 5581), respectively).

A further compound in this series was synthesized, that being the derivative in which the 2' and 3' hydroxy groups were unmodified. This was considered an attractive target since Canonico indicated that this CDS was effective in increasing survivability of animals intracranially inoculated with Japanese B encephalitis virus[38]. The derivatives were prepared according to Scheme III. The 2',3'-0-acetonide of ribavirin (2) was esterified with nicotinic anhydride in pyridine to give the 5'-nicotinate (6) which was subsequently deprotected with 88% formic acid at room temperature for 10 hours. The unprotected 5'-nicotinate (28) was then methylated to give the trigonellinate salt (29) and reduced in aqueous basic sodium dithionite to give the dihydrotrigonellinate (30, AVS 5582).

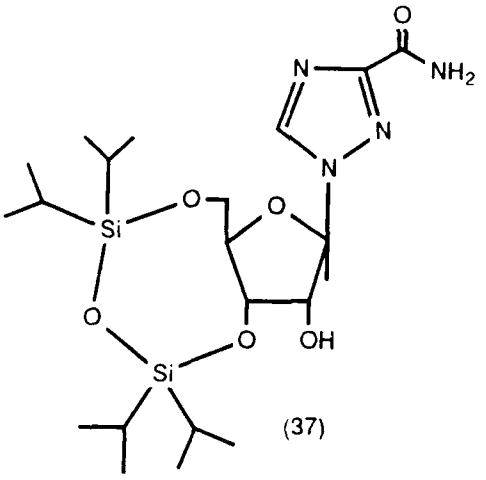
After, initial animal results indicated significant antiviral activity associated with the acetonide based CDS (5), a series of compound was prepared based on this prototype. The first of these is given in Scheme IV. The 2',3'-0-cyclopentylidene derivative of ribavirin (31) was obtained by treating ribavirin with cyclohexanone in the presence of mesitylene sulphonic acid. The obtained cyclic ester was then reacted with nicotinic anhydride to give the 5'-nicotinate (32). This ester was then quaternized with methyl iodide to give the trigonellinate salt (33) and reduced in basic aqueous sodium dithionite to give the 5'-based CDS (34) in high yield. This cyclopentylidene derivative is chemically much less stable than the corresponding acetonide and should more rapidly release the active riboside <u>in vivo</u>. Other derivatives which are presently being prepared include the cyclic carbonate (35).



(35)

2'-Based Chemical Delivery Systems

Initial work aimed at the preparation of ribavirin derivatives substituted in the 2'-position with 1,4-dihydrotrigonellinate moiety was based on the experiences of Ishido[39]. He reported that regioselective 2'-0-deacylation of peracylated purine and pyridine ribonucleosides could be effected via the action of hydrazine hydrate. This approach would be advantageous in that it would circumvent the problems of extensive protective and deprotective synthetic routes and allow selective manipulation at the desired site. 5',3',2'-tri-0-benzoyl ribavirin (36) was, therefore, prepared by acylating ribavirin with benzoic anhydride. Unfortunately, hydrazinolysis of the tribenzoate proved not to be selective. Efforts then shifted to selective 3',5'-protection through the use of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl). Reaction of ribavirin with this derivative gave rise to the 3',5'-0-(tetraisopropyldisiloxane-1,3-diyl) derivative (37) in high yield.



(37)

The next step, nicotinoylation of the protected riboside proved to be difficult. Acylation could not be achieved in more than 10% yield with either nicotinic anhydride in pyridine (1 to 6 equivalents) or in refluxing methylene chloride containing triethylamine. When the reaction was carried out at room temperature for 18 h in the presence of nicotinoyl chloride hydrochloride in pyridine, the starting materials disappeared but the intra-red spectrum of the products indicated dehydration of the amide to a cyano group (38). The

2'-nicotinate of the 3,5'-protected ribavirin derivative (39) was finally obtained in 90% yield by careful manipulation of reaction conditions. Removal of the protective disiloxyl group by previously published procedures lead to a complex mixture of products causing us to abandon this route[40].

Derivatives containing a nicotinate ester in the 2'-position were finally obtained using methods involving nonselective acylation of ribavirin followed by chromatographic separation of the isomers formed (Scheme V). This approach is more completely described in the next section. At any rate, 5'-dimethyoxytritylated ribavirin was reacted with benzoyl chloride at 0°C to yield a mixture of three products, the bis benzoate of the protected ribavirin (40), the 2'-benzoate and the 3'-benzoate of the protected riboside ((41) and (42), respectively). The crude mixture was subsequently subjected to nicotinoylation using nicotinic anhydride to yield a mixture of the bis benzoate (40), the 2'-benzoate, 3'-nicotinate (43) and the 2'-nicotinate, 3'-benzoate derivatives of the protected ribavirin (44). These materials were separated by open column chromatography on silica gel to yield the pure components. Deprotection of the bis ester followed by quaternization and reduction should give the first 2'-based CDS.

3'-Based Chemical Delivery System

The preparation of 3'-based CDS was first approached by using published procedures which indicated that the 2'-hydroxy group of a 5'-protected riboside could be selectively silated using t-butyldimethylsilyl chloride in the presence of nitrate ion[41,42]. Two reaction sequences were attempted using this technology, one in which ribavirin is subjected to selective silation to yield ostensibly the 5',2'-protected base and one in which the 5'-dimethoxytrityl derivative of ribavirin is used as the substrate to give rise to the 2'-derivitized material (45). In both instances, the procedure used did not give selectivity with both the 2'- and 3'-isomers being formed in nearly equal proportions.

Failing in the selective route, it was next decided to nonselectively acylate the 5'-protected riboside with nicotinic anhydride and separate the anticipated isomers chromatographically. When 5'-dimethoxytrityl ribavirin was reacted with 3 equivalents of nicotinic anhydride, a single product was formed, presumably the bis acylated protected riboside (46). When one equivalent of the anhydride was used, starting material as well

as the compound or mixture of compounds possessing a similar R_f value to the bis nicotinate earlier described. Initially, speculation pointed to 2'-acylation as earlier work on dimethoxytrityl substituted ribosides suggested that selective reaction at the 2'-position occurred in various synthetic manipulations[43]. This was further studied. 5'-Dimethoxytrityl ribavirin was treated with one equivalent of nicotinic anhydride and the reaction monitored hourly by thin layer chromatography (TLC). The reaction observed was estimated to have a half-life of 3 hours and was essentially complete after stirring at room temperature overnight. Attempts to isolate the products lead to degradation. Similar synthetic approaches were followed using benzoic (47) and anisic (48) anhydride.

Given the analytical and synthetic complexities of these nonselective acylations, an unambiguous method of structure determination was required. To this end, high field ^1H, ^{13}C and two dimensional ^1H-^1H (COSY) NMR techniques were selected. To provide an adequate base line for examining substitution on the ribavirin nucleus, a two-dimensional proton-proton experiment was performed in a d_6-DMSO solvent. The results of those determinations are presented in Figure 1 which include ^1H and ^{13}C resonances. A sample spectra is provided in Figure 2.

In the synthesis, careful manipulation and augmentation of earlier methods allowed for preparation of the desired isomer in various compositions. When, for example, 5'-dimethoxytrityl ribavirin was treated with benzoyl chloride at 0°C and the reaction mixture was left overnight, three fractions were observed which could be separated and purified by open column chromatography (Scheme V). These fractions included the 2',3'-dibenzoate derivative of 5'-dimethoxytrityl ribavirin (40), a mixture of monobenzoylated products and a single, pure monobenzoate. When the synthetic procedure was repeated but with shorter reaction times, the dibenzoate formed in much lower quantities and the two isomeric monobenzoates could be obtained in pure form by open column chromatography. Both isomers were obtained as white solids. Two-dimensional ^1H NMR indicated that the less polar isomer was the 2'-benzoate derivative of the protected riboside (41) and that the more polar isomer was the 3'-benzoate (42). Interestingly both of these purified isomers underwent 0-to-0 transacylation in DMSO to produce product scrambling as shown in Figure 3. In any case, the 3'-benzoate derivative was cleanly acylated with nicotinic anhydride in the presence of a nucleophilic

catalyst (DMAP) to yield the 5'-dimethoxytrityl ribavirin 2'-nicotinate 3'-benzoate in good yield (49).

Further work with this reaction scheme indicated that the chromatographic separations could be reduced by treating the isomeric mixture of dimethoxytritylated ribavirin benzoates with nicotinic anhydride to yield the 2',3'-dibenzoate of the protected riboside (40), and the 2'-nicotinate-3'-benzoate (44) and 2'-benzoate-3'-nicotinate (43) derivatives. Open column chromatography conveniently separated these 3 fractions in a ratio of 1.2 to 2.8 to 1.0, respectively. The 2'-nicotinate-3'-benzoate protected riboside (44) was then deprotected in the presence of 80% acetic acid to yield the 5'-hydroxy derivative (50). This material was then methylated with methyl iodide to give the 2'-trigonellinate derivative (51) and reduced in aqueous dithionite to give the 2'-based CDS (52, AVS 5756).

The use of other masking esters, the derivitization of the 5'-position and other manipulations are currently being considered.

2',3'- and 2',3',5'-Based Chemical Delivery Systems

Attachment of the brain-targeting 1,4-dihydrotrigonellinate derivative to several hydroxy groups was considered. In the first such system, ribavirin was percylated with nicotinic anhydride in dry pyridine to yield the trinicotinate (53)(Scheme VI). This tris ester was quaternized with methyl iodide to give the tris trigonellinate salt (54) and reduced in aqueous sodium dithionite to give the 2,'3',5'-tris(1,4-dihydrotrigonellinate) derivative of ribavirin (55). The 2',3'-dinicotinate of ribavirin was prepared by treating 5'-dimethoxytritylated ribavirin (7) with nicotinic anhydride. The resulting bis ester (56) was then quaternized giving the 2',3'-bis trigonellinate salt and reduced to give the 2',3'-CDS.

Derivatization of the Carboxamide of Ribavirin

The carboxamide functionality of ribavirin was considered as a possible synthetic handle. Initial manipulations were aimed at the formation of hydroxymethyl amides which would allow for esterification but would also provide for rapid reversion of the derivative to the starting amide upon hydrolysis. Ribavirin triacetate and tribenzoate

(36) were first considered. Hydroxymethylation of the tribenzoate could not be effected using formaldehyde in basic conditions at room temperature or at 60°C. In addition there was no reaction between paraformaldehyde and ribavirin tribenzoate at 40°C in the presence of sodium methoxide. At elevated temperatures polymerization appears to occur in this reaction as indicated by NMR. Further attempts to hydroxymethyl ribavirin triesters included the use of base catalysts such as potassium carbonate, ammonium hydroxide and potassium hydroxide, sodium methoxide and triethylamine, none of which facilitated the reaction. The poor solubility of the ribavirin triester in aqueous solutions prompted a series of nonaqueous reactions including hydroxymethylation by paraformaldehyde or 1,3,5-trioxane in carbon tetrachloride, dichloroethane and tetrahydrofurane. No reaction occurred in the temperature range between 25°C-40°C and polymerization consistently occurred at refluxing temperatures. Use of acid catalysts resulted in amide dehydration. In addition to formaldehyde, more electrophilic aldehydes were considered. Unfortunately no reaction occurred between tribenzoyl ribavirin and chloral hydrate, anhydrous chloral and n-butyl glyoxylate[44]. Similar failures were reported for ribavirin tripropionate (57). Other derivatives of the amide position which were not explored include thioamides, amidoximes, O-acylamidoximes, 1,2,3-oxadiazoles, amidines, and ureas.

While the amide substituent was resistant to alkylation, it could be acylated. Two such derivatives were prepared as indicated in Schema VII and VIII. As illustrated in Scheme VII, dimethyoxytritylated ribavirin (7) can be treated with an excess of isobutyric anhydride to yield the 2',3',N'-triisobutyrate derivative of the 5'-protected riboside (58). Subsequent detritylation yields (59) which is then nicotinoylated to give the 5'-nicotinate (60). Quaternization yields the 5'-trigonellinate (61) and reduction gives rise to the 5'-dihydrotrigonellinate derivative of ribavirin acylated in the 2',3' and N'-positions (62, AVS 5222). In Scheme VIII, careful manipulation of reaction conditions allows the formation of the 5',N'-dinicotinate derivative of ribavirin 2',3'-diacetate (64) when ribavirin 2',3'-diacetate (63) is treated with nicotinic anhydride. The tetracylated product (64) is then quaternized to give the 5',N'-bis trigonellinate salt (65). Reduction of (65) will give the 5',N'-bis(1,4-dihydrotrigonellinate)-2',3'-diacetate.

In all cases, compounds have been scaled up from a few hundred milligrams to 2-5 g amounts.

Analytical Methodology

One of the most challenging aspects of this project has been the development of hardy and reliable analytical techniques to detect, separate and quantitate the ribavirin-CDS's, the corresponding quaternary salts and ribavirin itself, both in vitro and in vivo. Ribavirin is highly water soluble and does not have a chromaphore which significantly absorbs light above 208 nm. The ribavirin quaternary salts were shown to act in a chromatographically distinct manner compared to previously prepared trigonellinate salts thus requiring the development of novel HPLC methods. This discussion will concentrate on each component of the CDS starting with the relatively well behaved dihydronicotinates, progressing to the trigonellinate salts and ending with the various attempts which have been made to quantitate the parent compound, ribavirin, and ribavirin derivatives.

The first CDS studied was the 2',3'-0-acetonide-5'-dihydrotrigonellinate derivative of ribavirin (5). This compound has the usual dihydropyridine (Band III) absorbance around 360 nm. Several HPLC systems have been developed to quantitate this derivative including the following:

1)	Column:	2 Perkin-Elmer HS-3 C18 columns (3 μm particle, 3.3 cm x 4 mm i.d.) in series
	Mobile Phase:	Methanol:0.02 M KH_2PO_4:H_2O 40:40:20
	Flow Rate:	1.0 mL/min
	Temperature:	Ambient
	Detection:	UV, 360 nm
	Retention Time:	11.0 min
2)	Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.4 mm i.d.)
	Mobile Phase:	Methanol:0.008 KH_2PO_4 40:60
	Flow Rate:	0.4 mL/min
	Temperature:	21.8°C
	Detection:	UV, 360 nm
	Retention Time:	10.0 min

Each of these systems could also isolate and quantitate the corresponding 5'-nicotinate-2',3'-0-acetonide and the 2',3'-0-acetonide derivatives of ribavirin. The retention times of these species was in system 1, 10 min and 6.3 min, respectively, and 10 min and 5.2, respectively, in system 2. The trigonellinate salt (4) could not be eluted using these systems.

Assay conditions similar to System 2 were utilized for analysis of the 5'-1,4-dihydrotrigonellinate derivatives of ribavirin-2',3'-dibenzoate (25), ribavirin-2',3'-dipivaloate (24), ribavirin-2',3'-diisobutyrate (26) and 2',3'-N'-triisobutyrate-5'-1,4-dihydrotrigonellinate ribavirin (62) as described below.

Compound (25)

Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.1 mm i.d.)
Mobile Phase:	Methanol:0.008 M KH_2PO_4 (58:42)
Flow Rate:	0.4 mL/min
Temperature:	21.8°C
Detection:	UV, 360 nm
Retention Time:	17.8 min

Compound (24)

Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.1 mm i.d.)
Mobile Phase:	Methanol:0.008 M KH_2PO_4 (60:40)
Flow Rate:	0.4 mL/min
Temperature:	Ambient
Detection:	UV, 360 nm
Retention Time:	13.0 min

Compound (26)

Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.1 mm i.d.)
Mobile Phase:	Methanol:0.008 M KH_2PO_4 (58:42)
Flow Rate:	0.4 mL/min
Temperature:	Ambient
Detection:	UV, 360 nm
Retention Time:	6 min

Compound (62)

Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.1 mm i.d.)
Mobile Phase:	Methanol:0.008 M KH_2PO_4 (58:42)
Flow Rate:	0.4 mL/min
Temperature:	Ambient
Detection:	UV, 360 nm
Retention Time:	15 min

In various instances, these systems were modified depending on the specific analysis. When, for example, the 2',3'-diisobutyryl-5'-dihydrotrigonellinate derivative of ribavirin (26) was examined in biological tissues, the following conditions were used:

Column:	Spheri-5 RP-8 column (Brownlee) Fitted with a C-8 guard column
Mobile Phase:	Acetonitrile:0.067 M KH_2PO_4 (75:25)
Flow rate:	1 mL/min
Temperature:	Ambient
Detection:	UV, 360 nm
Retention Time:	5 min

In this case, detection at 266 nm greatly increased sensitivity due to the higher extinction coefficient at this absorbance. No interferences were observed using this lower wavelength.

The above listed chromatographic systems were then used to assay the stability of several CDS's as illustrated in Table I. The rate of degradation of each of four systems was assayed in rat blood, brain homogenate, liver homogenate and in some cases phosphate buffer. The acceleration of degradation rates in biological matrices compared to buffers is consistent with an enzymatically-mediated decomposition. Of the compounds examined, the acetonide appears to be the most stable under the conditions utilized. These chromatographic systems have been used in various in vivo tissue distribution studies which will be discussed in the next section.

The development of stable chromatographic systems for the trigonellinate salts has been problematic. Initial studies indicated that the 2',3'-acetonide derivative of 5'-1,4-dihydrotrigonellonyl ribavirin (4) eluted poorly if at all on ODS (C18) or C8 silica stationary phases. Inclusion of buffers, anionic ion pair reagents and cationic competitive quaternary ammonium salts did not improve the chromatography. A non-

aqueous reverse phase system based on a cyano derivitized column gave better retention and relatively good peak shape. This system is described below:

Column:	Spherisorb CN (5 μm particle size, 250 mm x 4.6 mm i.d.)
Mobile Phase:	Acetonitrile + 0.002 M tetrabutylammonium perchlorate
Flow Rate:	2.0 mL/min
Temperature:	Ambient
Detection:	UV, 266 nm
Retention Time:	9.8 min (19.7 min at 1 mL/min)

Unfortunately this system was found to be unstable and was associated with relatively rapid degradation of the column leading to irreproducibility. A second system was thus developed:

Column:	Spherisorb C-8 (Brownlee Cartridge, 10 cm x 2.1 mm i.d.)
Mobile Phase:	Isopropanol:0.05 M KH_2PO_4 (10:90 + 0.005 M sodium octanesulfonate)
Flow Rate:	0.4 mL/min
Temperature:	Ambient
Detection:	UV, 266 nm
Retention Time:	7 min

This system was useful, however, only for limited <u>in vitro</u> and bulk compound purity studies. It was not sufficiently stable to be used for analysis of the quaternary salts in biological tissues due to the appearance of interfering peaks and the poor extractability of the trigonellinate salts from biological homogenates.

Finally a reproducible method was developed for the 5'-trigonellinate of ribavirin acetonide. This method employed a C1 column and is summarized below:

Column:	Spherisorb C1 (25 cm x 4.6 mm i.d.) fitted with a C2 guard column
Mobile Phase:	Acetonitrile:0.01 M acetate buffer pH 4.4 (50:50)
Flow Rate:	1 mL/min
Temperature:	Ambient
Detection:	UV, 224 nm
Retention Time:	16.2 min

This system allowed for both chemical studies of the 5'-trigonellinate of the ribavirin acetonide as well as in vitro and in vivo investigation.

Several physiocochemical parameters of (4) were measured including the pH of maximum stability of the 5'-trigonellinate of ribavirin acetonide, its stability in acetonitrile, its partition coefficient and its stability in buffer, blood and brain homogenate. The pH of maximum stability was determined using the following equation

$$[H^+]_{min} = \left(\frac{k_{OH^-} \cdot Kw}{k_{H^+}} \right)^{1/2}$$

when k_{OH^-} is the specific catalytic base rate constant, k_{H^+} the specific acid catalytic rate constant and Kw, the ionization constant for water. In this equation k_{OH^-} and k_{H^+} can then be obtained from the following relationships:

$$\log k_{OH^-} = \log k + pKw - pH$$

and

$$\log k_{H^+} = \log k + pH.$$

Using the rate constants obtained from degradation of (4) in 0.1 M HCl and phosphate buffer (pH 6.47 and 7.57) the specific acid and base catalytic constants were obtained (Table 11) and the pH_{min} was found to be 3.92 at 25°C. At this pH, the trigonellinate should have a $t_{1/2}$ of approximately 3.5 months as estimated using the equation

$$k_{min} = 2(K_{H^+} k_{OH^-} Kw)^{1/2}$$

This indicates that at a pH of 4, solutions of the acetonide (4) should be stable. The degradation product in acidic media was exclusively ribavirin 5'-trigonellinate while in basic media, only ribavirin acetonide was detected.

Since acetonitrile is used both in the preparation of biological tissues for analysis and in HPLC mobile phases, the stability of ribavirin 5'-trigonellinate 2',3'-acetonide in this solvent was considered. The ribavirin trigonellinate was shown to degrade in acetonitrile in a zero order process with an apparent rate constant of $k = 3.9 \times 10^{-10}$ M/sec. As this process is zero order, the half-life of disappearance of this material will depend on its initial concentration. The major product of degradation in these studies was ribavirin acetonide.

The efficient extraction of the CDS components is essential for accurate and precise quantitations. The distribution constant (concentration in acetonitrile/concentration in the aqueous phase) were therefore determined for ribavirin acetonide (2), ribavirin 5'-trigonellinate (28) and ribavirin 5'-trigonellinate-2',3'-acetonide (4). These data are collected in Table III. As shown, the extraction is pH independent and the efficiency range from 59% in the case of the ribavirin acetonide (2), to 32% in the case of the acetonide trigonellinate (4) to 0% for ribavirin trigonellinate (28).

The in vitro stability of the ribavirin 5'-trigonellinate-2',3'-acetonide was assayed in several biological matrices and phosphate buffer are shown in Table IV. As indicated whole rat blood degrades the trigonellinate about twice as rapidly as does phosphate buffer or brain homogenate.

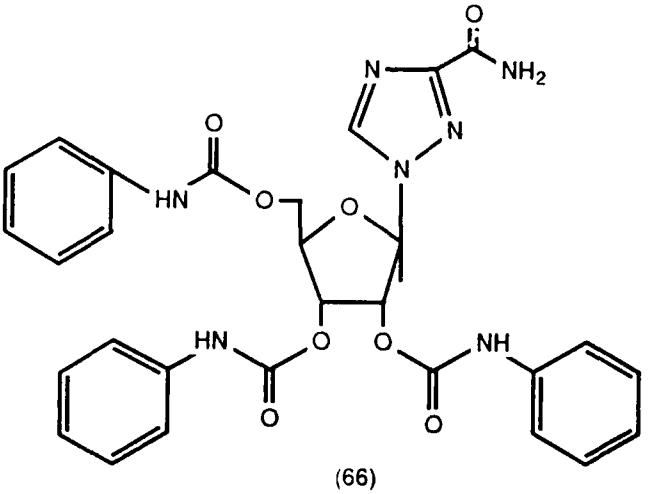
A second trigonellinate salt which has been examined is ribavirin 5'-trigonellinate-2',3'-diisobutyrate (26). The assay of this material was accomplished using methodologies similar to those employed for (4). The tissue distribution of this species in rats after i.v. administration of the ribavirin 5'-(1,4-dihydrotrigonellinate)-2',3'-diisobutyrate is discussed in the in vivo section.

Analytical system for examining ribavirin itself have been considered. Published methods include the use of phenyl boronate affinity chromatography and extraction/ion exchange chromatography both of which are used as a sample clean-up procedure prior to HPLC determinations[45,46]. These methods are time consuming and are not convenient. In deference to these protocols, attempts have been made to develop online systems which do not require extensive sample manipulation prior to assay.

Three HPLC stationary phases were considered including a Partisil PAC, Spherisorb CN and Spherisorb Phenyl support. In the Partisil column operating with a mobile phase consisting of 85% aqueous acetonitrile, ribavirin could be eluted at 7.7 min but the peak shape was unacceptably poor. Using the Spherisorb CN column with aqueous acetonitrile (92% acetonitrile) ribavirin gave a retention time of 5.14 min but the sensitivity of the system was low. The use of gradient elution did not significantly improve this finding. The Spherisorb column appeared to be the most useful. Using a 20% aqueous acetonitrile mobile phase, ribavirin eluted at 3.2 min and linear standard

curves could be generated. Unfortunately, this analysis would require extraction of the analyte into an organic solvent and due to the poor lipophilicity of ribavirin, this process is not efficient. In an effort to improve the selectivity of this determination, a different type of detection was examined. Specifically, ribavirin was chromatographed on an anion exchange column (Carbo Pac PA-1, Dionex®) using a 0.1 M NaOH mobile phase at a flow rate of 1.0 mL/min. The drug was then detected using a pulsed amperometric detector (Dionex®) in which the potential was set at 0.05 and the sensitivity at 100 nA. Under these conditions, the retention time for ribavirin was 6.5 min and its limit of detection in water was 100 μg/mL. The sensitivity of this assay could be increased approximately 3-fold by addition of 0.02 M sodium acetate to the mobile phase. Unfortunately, blank brain samples gave large chromatographic responses which covered ribavirin.

An alternate attempt to generate a useable analytical system for ribavirin involved precolumn derivitization of the riboside. The specific modification used was percarbanilate (66) formation achieved by reaction of ribavirin with phenyl isocyanate. This method did not prove to be useful due to the extensive reaction conditions and clean-up required and the low yield of the reaction.



(66)

Given these attempts, a decision has been made to use published procedure although this will severely restrict the number and the tissues which can be examined[45].

Thus far the published methods have only been applied to relatively "clean" samples including plasma and lung tissue.

Animal Toxicology Studies

An acute i.v. paradigm was used to access the toxicity of the CDS's prepared. In screening compounds for toxicity, the dose was dictated by the maximum solubility of a particular dihydrotrigonellinate in dimethylsulfoxide (DMSO), the vehicle selected for these studies. After solubility determination, animals were given a DMSO solution containing 90% of the maximum solubility value. If death or overt toxicities ensued, the dose was systematically lowered. General robustness, body weight, survival and gross organ appearance at necropsy were then monitored. Table V summarizes the lethality resulting from various doses of CDS's used in this study. As illustrated, the 5'-(1,4-dihydrotrigonellinate)-2',3'-diisobutyrate derivative of ribavirin was the compound found to possess the lowest degree of acute toxicity. The dibenzoate (25) and 2',3',N'-tributyrate derivatives (62) appeared to be the most toxic. In the case of the dibenzoate, a dose of 34 mg/kg caused cyanosis prompting a diminution of the dose. The dipivaloate (24) produced some ataxia at the 57.3 mg/kg dose level so the dose was lowered to 38.2 mg/kg. At this dose no toxic effects were observed. In all cases, toxic doses of compound were associated which darken lungs at necropsy. Body weight changes were used as a general indication of toxicity. Those data are shown in Table VI. The animals used in these studies were adults characterized by relatively stable body weights. Dramatic losses in mass were noted and correlated with the dose of the compound given. Some individuals lost significant amounts of weight and some animals tended to gain weight more slowly. The most toxic compound in this required approach to be the 2',3',N'-tributyrate (62) while the diisobutyrate (26) and dipivaloate (24) were relatively nontoxic. The low toxicity exhibited by the diisobutyrate promoted this material to the first compound to be examined for in vivo distribution.

In Vivo Tissue Distribution

The diisobutyrate (26) was administered i.v. to conscious restrained Sprague-Dawley rats (BW=200-250 g) in a DMSO vehicle. At 15 min, 1, 2, 6 and 24 hours post-

drug administration animals were sacrificed by rapid decapitation and brain and blood collected and frozen. These simply were later weighed, homogenized and extracted with acetonitrile. The acetonitrile layer was then assayed for both the CDS and the corresponding trigonellinate. No CDS was detected in any tissue at any time. This is not unusual given the metabolic instability of the CDS and its large volume of distribution. In addition, the ribavirin 2',3'-diisobutyrate-5'-trigonellinate (22) was not detected in blood presumably due to rapid hydrolysis. In the brain, however, the "intact" pyridinium salt (22) was detected through 2 hours (disappearing by 6 hours). The concentration of the quaternary salt in brain is summarized in table VII. The levels of the CDS metabolite rose to a maximum at 1 hr before decreasing in concentration. In addition to the quaternary salt an unknown metabolite appeared at 8.27 min which was neither ribavirin nor the ribavirin 2',3'-diisobutyrate. This peak did not appear to be influenced by ion pairing reagents suggesting that it is not charged. This metabolite was present in low concentration initially, rose to its maximum level at 2 hours and then fell. Attempts are being made to identify this material. The improved analytical methodologies will allow more selective and sensitive examination of the CDS in vivo.

Antiviral Activity Studies

Prepared compounds were submitted to Dr. Robert Sidwell at Utah State University for antiviral screening. The model used was an in vivo murine system in which animals were inoculated intercerebrally with the Balliet strain of Punta Toro virus[37]. This is a *Phlebovirus* of the *Bungaviridae* family. It is closely related to the pathogens responsible for phlebotomus, or flea sandfly, fever and Rift Valley fever[37]. The particular strain of this virus causes a deadly encephalitis in which i.v. administered ribavirin has no perceptible effect. Specifics of this model have been published. Table VIII gives the initial results obtained for compounds (24), (25), (5) and (26). As illustrated, significant extension of the animal's life span is observed in several cases and the acetonide (5) significantly increases the number of survivors relative to vehicle controls. In these animals, the drug was administered 4 hours prior to viral inoculation.

Subsequent reports, however, contradicted these initial findings and suggested that

the compound was not active at least after a single dose in the model examined. Work is currently being directed to retest the previously submitted compounds using a multiple dose paradigm. Three newly prepared compounds will shortly be sent for testing. In addition to the compounds present in Table VIII, the tributyrate derivative (62) and ribavirin acetonide (2) were examined for antiviral activity but were found to be inactive.

Discussion

Several synthetic routes were utilized in the preparation of CDS for ribavirin. The 5'-based carriers could be conveniently prepared by first selectively alkylating the 5'-position using dimethoxytrityl chloride, acylating the unmanipulated 2' and 3'-hydroxy groups and subsequently deprotecting the riboside. The free 5'-functionality was then nicotinoylated, quaternized and reduced to yield the desired derivatives.

The carrier system based on the 2' and 3'-hydroxylic groups were not as accessible. Initial attempts were made to introduce various groups selectively to the ribavirin nucleus using various previously published methods. Unfortunately, specific acylation could not be performed and as a result, nonselective method followed by chromatographic purification were necessary. By careful manipulation of the reaction conditions, a preponderance of the 2'/3'-monoacylated products could be obtained which were then either separated by open column chromatography or subsequently acylated and separated. This latter method was typically followed as the 2'/3'-monoacylated products were found to undergo 0-to-0 transacylation in solution. This equilibrium resulted in product scrambling. While 2' to 3' and 3' to 2' exchange occurred, no migration of moiety acylated at these secondary positions to the 5'-hydroxy group occurred.

Derivitization of the amide group did not prove to be feasible. Attempts to hydroxymethylate this position resulted in either no reaction or, at higher temperature, polymerization. Other electrophiles such as anhydrous chloride or alkyl glyoxylates were similarly unreactive towards this functionality. Interestingly, the carboxamide was relatively sensitive to dehydration resulting in the formation of a nitrile substituent. The lability of this group to the usually difficult reaction may be related to the adjacent ring

nitrogen. This proton acceptor may facilitate the described degradation by mechanisms described in Scheme IX.

After preparation, analytical methodologies were developed to accurately and precisely separate, detect and quantitate the CDS's and their precursor/metabolites. The lipophilic dihydrotrigonellinates could be easily followed using traditional reversed phase techniques. The trigonellinate salts however proved to be unusually in their chromatographic properties relative to other systems which have been studied. After examining several possibilities, the best results were obtained using a C-1 silica based column. The water soluble riboside, ribavirin, is difficult to assay using HPLC and was not adequately eluted using either normal or reversed phase systems. Electrochemical detection did not improve the selectivity of the determinations. Phenyl bororate affinity chromatography and other approaches used preparatory to chromatographic separation are presently being validated.

In vitro studies indicated that the CDS rapidly degrades in homogenates of various tissues. Most of the compounds tested are relatively nontoxic when administered acutely and have, therefore, been examined in various in vivo models. Administration of one of the CDS's (26), resulted in brain uptake of the CDS with subsequent conversion to the trigonellinate salt. Antiviral activity studies are in progress.

EXPERIMENTAL SECTION

Microcombustion analysis of compounds synthesized was performed by Atlantic Microlabs, Atlanta, GA. Uncorrected melting points (m.p.) were determined with either an Electrothermal or Thomas-Hoover melting point apparatus. Ultraviolet spectra (UV) were obtained on either a Hewlett-Packard 8451A diode array or a Shimadzu U rapid scan spectrophotometer. Infra-red spectra (IR) were recorded on a Beckman Microlab 620 MX spectrophotometer. Samples were analyzed as potassium bromide pellets or as a thin film on sodium chloride windows. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on either a Varian EM 360 or a Varian XL 200 (200 MHz, FT mode) and ^{13}C samples obtained on the latter instrument (50 MHz, FT mode). Samples were dissolved in an appropriate solvent and chemical shifts (δ) reported relative to tetramethylsilane in the case of ^{1}H-NMR and referenced to the

central signal $CDCl_3$ (77 ppm) or d_6-DMSO (39.5 ppm) in the case of ^{13}C-NMR. Thin-layer chromatography was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with indicated silica gel (60 mesh). Ribavirin was obtained from Viratec, Inc. and other solvents and reagents from either Aldrich or Sigma Chemical Company. HPLC was performed using a Spectra-Physics SP8800 pump, an SP8490 UV-Vis variable wavelength detector, an SP8780 refrigerated autosampler and a SP4270 integrator. All chromatographic operations were controlled via an IBM-AT microprocessor using Chromnet® software.

1-(2',3'-0-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (2, AVS 5221)

The synthesis of Cpd. 2, using the method described by Witkowski and Robins[4] resulted in an intractable tar. In this method, ribavirin was treated with a mixture of 70% perchloric acid.

Cpd. 2 was synthesized in a manner similar to that described for imidazole nucleosides. Dry hydrogen chloride (7.4 g) was dissolved in acetone (160 mL). To this solution ribavirin (5.0 g, 20.5 mmol) was added and the mixture was stirred at RT for 24 h. It was then poured into a stirred cold solution of ammonium hydroxide (27 mL) in water (240 mL). After a pH 8 solution was obtained, it was concentrated to a small volume (100 mL). The separated ammonium chloride was removed by filtration, the filtrate was evaporated under reduced pressure and the residue was dried in vacuo at 50° for 2 h. The dry residue was repeatedly extracted with warm chloroform. Evaporation of the chloroform under reduced pressure gave the product (5.3 g, 91%) as a white solid.

Tlc silica plates; BuOH:AcOH:H_2O 4:1:1; $R_f = 0.67$

IR (nujol mull): υ_{NH+OH} 3380 and 3180; $\upsilon_{C=O}$ 1700, $\upsilon_{C=N}$ 1670; $\upsilon_{C=O}$ 1130-1080.

^1H-NMR (DMSO-d_6): δ 8.9(s,1H,triazole proton), 7.8(br d, NH_2), 6.25(d,1H,1'H), 5.15(m,3H,5'OH + 2'H +3'H), 4.3(t,1H,4'H), 3.5(2H,CH_2), 1.4(2s, 6H, 2xCH_3).

1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-0-isopropylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (4)

Cpd. 2 (2.0 g, 0.007035 mol) was dissolved in anhydrous dimethylformamide (80

mL) and to it were added N,N-dimethylaminopyridine (0.26 g, 0.00213 mol) and trigonelline anhydride (3) (3.6 g, 0.00703 mol). The reaction mixture was stirred overnight at room temperature. The dimethylformamide was removed in vacuo at low temperature and the resulting oil was dissolved in dry acetone. After 2 h, a solid precipitated out. This was removed by filtration and the filtrate was evaporated to dryness in vacuo. The resulting yellow solid was recrystallized from ethanol to give the product (Cpd. 4) (1.3 g, 36%).

IR (nujol mull): υ_{NH} 3580, 3440, 3240, 3160, $\upsilon_{C=O}$ 1730, 1690, $\upsilon_{C=N}$ 1645, $\upsilon_{C=C}$ 1600, υ_{C-O} 1180, 1120, 1070.

^{1}H-NMR (DMSO-d_6): δ 9.6(s,1H,Pyr), 9.3(d,1H,Pyr), 9.0(d+s,2H,Pyr + triazole proton), 8.3(m,1H,Pyr), 7.7(br d,2H,NH_2), 6.35(s,1H,1'H), 5.3(2H,2'H + 3'H), 4.6(m,6H,CH_2 + N-CH_3 + 4'H), 1.5(2s,6H,2xCH_3).

UV λ_{max} (MeOH): 266, 217.

1-[5'-(1-methyl-3-carbonylpyridinium)(2',3'-0-isopropyldene)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (4a)

Cpd. 6 (6.0 g, 15.4 mmol) was dissolved in 70 mL dry acetone and 8 g of methyl iodide were added. The mixture was refluxed for 24 h after which the solvent was removed in vacuo and the resulting glass was powdered with a pestle and mortar. It was washed with a small amount of acetone followed by ether and dried in vacuo. This gave 8 g (97.7%) of product as a bright yellow solid.

IR cm^{-1} (nujol mull): υ_{NH} 3440, 3300, 3240, 3180, $\upsilon_{C=O}$ 1735, 1680, $\upsilon_{C=C,C=N,}$ NH_2 def 1640, 1590, υ_{C-O} 1080, 1110.

^{1}H-NMR (DMSO-d_6): δ 9.65 (1H,s,pyrH), 9.45 (1H,d,pyrH), 9.15 (1H,d,pyrH), 9.05 (1H,s,5-H), 8.45 (1H,m,pyrH), 7.7-7.9 (2H,2bs,NH_2), 6.45 (1H,s,1'-H), 5.4 (2H,bs,2'-H,4'-H), 4.75 (3H,bs,5'-CH_2,3'-H), 4.6 (3H,s,N-CH_3), 1.45-1.65 (6H,2s,2xCH_3).

U.V. λ_{max} (MeOH): 266, 217.

1-[5'-(methyl-1,4-dihydronicotinoyl)-1-(2',3'-0-isopropylidene-β-D-ribofuranosyl)]-1,2,4-triazole-3-carboxamide (5, AVS 5505)

To a stirred, degassed, ice-cold deionized water (4 mL) solution of Cpd. 4 (0.05 g,

0.000097 mol), a mixture of sodium bicarbonate (0.033 g, 0.000288 mol) sodium dithionite (0.062 g, 0.000288 mol) was added. The reaction was maintained at 0°C and under argon. After 3.5 h the precipitated yellow solid was filtered and washed with cold water and cold ethyl acetate. It was dried in vacuo and 0.02 g (53%) of the dihydro was obtained.

UV λ_{max} (MeOH): 360, 211.

^{1}H-NMR (DMSO-d_6): δ 8.75(s,1H,triazole), 7.65(br d, 2H,NH_2), 6.85(s,1H,Pyr-C_2), 6.25(s,1H,1'H), 5.75(d,1H,Pyr-C_6), 5.05(m,1H,Pyr-C_5), 4.85-4.3(m,3H,2'H,3'H,4'H), 4.15(m,2H,Pyr-C_4), 3.3(s,2H,5'-CH_2), 2.9(s,3H,N-CH_3), 1.45(2s,6H,2xCH_3).

1-[5'-(3-carbonylpyridine)-(2',3'-0-isopropylidene-β-D-ribofuranosyl)]-1,2,4-triazole-3-carboxamide (6)

2',3'-0-isopropylidene-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (20.0 g, 70.35 mmol) was dissolved in 400 mL anhydrous pyridine and cooled to 0°C. (32.1 g 240.7 mmol) Nicotinic anhydride was added portionwise and the reaction mixture was stirred at room temperature for 24 h. It was poured on to 1000 mL ice and extracted (2 x 1000 mL) with CH_2Cl_2. The combined organic extracts were washed with (2 x 700 mL) 5% $NaHCO_3$, (700 mL) H_2O, dried ($MgSO_4$) and the solvent was removed in vacuo. The resulting oil was purified on a silica column with $CHCl_3$: MeOH (9:1) as eluant. This gave 19.5 g (71.2%) of white solid product.

IR cm^{-1} (nujol mull): υ_{NH} 3440, 3330, 3260, 3180, υ_{C-H} unsat 3120, $\upsilon_{C=O}$ 1730, 1690, $\upsilon_{C=N}$, NH_2 def 1600, υ_{C-O} 1080, 1100.

^{1}H-NMR ($CDCl_3$): δ 9.25 (1H,s,pyr,H), 8.85 (1H,d,pyrH), 8.45 (1H,s,5-H), 8.3 (1H,m,pyrH), 7.5 (1H,m,pyrH), 7.25 (1H,bs,NH), 6.2 (1H,s,1'-H), 5.45(1H,t,4'H), 5.1(1H,d,2'-H), 4.4-4.9 (3H,m+s,3'-H,5'-CH_2), 1.45-1.65 (6H,2s,2xCH_3).

1-(5'-0-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (7)

10.0 g of ribavirin was dissolved in 300 mL anhydrous pyridine and the solution was cooled to 0°C. 34.0 g of Dimethoxytrityl chloride was added to it portionwise over a 2 h period and the mixture was stirred at room temperature for 24 h. 40 mL of methanol was added to it and the solvent was removed in vacuo. The resulting oily

solid was dissolved in 500 mL methylene chloride and washed with 5% $NaHCO_3$ (500 mL) water (500 mL), dried ($MgSO_4$) and the solvent was removed in vacuo. The resulting oily solid was washed with ether, filtered and purified on a silica column with $CHCl_3$:MeOH (10:1) as eluant. This gave 41.3 g (92.3%) of product was an off white solid.

IR cm^{-1} (nujol mull): $\upsilon_{NH,OH}$ 3460, 3200, $\upsilon_{C=O}$ 1690, $\upsilon_{C=C,C=N}$ 1610,2590.

^{1}H-NMR (DMSO-d_6): δ 8.873 (s,1H,5-H); 8.317 (s,1H,NH); 8,314 (s,1H,NH); 7.28 (m,9H,arom,J=8.8 Hz); 6.85 (dd,4H,arom,J=8.8 and 2.4 Hz); 5.973 (d,1H,1'-H,J=2.4 Hz); 5.680 (d,1H,2'=OH,J=5 Hz); 5.234 (d,1H,3'-OH,J-6.2 Hz); 4.453 (q,1H,2'-H); 4.376 (q,1H,3'-H); 4.1 (apparent q,1H, 4'-H); 3.730 (s,6H,2xOCH_2); 3.17 (m,2H,5'-CH_2) ^{13}C-NMR (DMSO) (75 MHz, FT mode) δ (ppm): 160.4(CO), 158.1, 158.15, 157.5, 145.6, 144.9, 135.7, 139.8, 129.7, 127.84, 127.8, 126.6, 113.2, 91.4, 85.4, 83.1, 74.1, 70.4, 63.6, 55.0.

1-(5'-0-dimethoxytrityl-2',3'-bis-0-pivaloate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (8)

Cpd. 7 (5.0 g) was dissolved in 25 mL dry pyridine and to it 0.5 g of N, N-dimethylaminopyridine and 9.3 mL of pivaloic anhydride were added. The mixture was stirred at room temperature for 24 h. It was then poured on a 200 mL ice and extracted (2 x 200 mL) with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$ (2 x 200 mL), H_20 (200 mL), and then dried ($MgSO_4$). The solvent was subsequently removed under reduced pressure. The resulting oil was purified on a silica column with $CHCl_3$:MeOH(40:) as eluent. This gave 4.6 g (70.3% of a white solid as product.

IR cm^{-1} (nujol mull): υ_{NH} 3480, 3340, $\upsilon_{C=O}$ 1740, 1710, $\upsilon_{C=N}$, NH_2 def 1610, 1585, υ_{C-O} 1130, 1160, 1180.

^{1}H-NMR ($CDCl_3$): δ 8.5 (1H,s,5-H), 7.7-6.7 (15H,m,atom ring,NH_2), 6.4-6.0 (2H,m+s,1'-H,2' or 3'H), 5.75 (1H,t,4'H), 4.45 (1H,m,2'-H or 3'-H), 3.9 (6H,s,2xOCH_3), 3.6 (2H,bs,5'-CH_2), 1.2 (18H,s,6xCH_3).

1-(5'-0-dimethoxytrityl-2',3'-bis-0-benzoate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (9)

Cpd. 7 (5.0 g) was dissolved in 25 mL anhydrous pyridine, to this were added 0.5 g N,N-dimethylaminopyridine and 10.4 g benzoic anhydride. The mixture was stirred at room temperature for 24 h. It was poured on to 200 mL ice and extracted (2 x 200 mL) with CH_2Cl_2. The combined organic extracts were washed with (2 x 200 mL) $NaHCO_3$, 200 mL H_20, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oil was chromatographed on a silica column with a $CHCl_3$:MeOH mixture 40:1 as eluant to give 5.5 g (80.3%) of product as white solid.
IR cm^{-1} (nujol mull): υ_{NH} 3470, 3340, $\upsilon_{C=O}$ 1730, 1700, $\upsilon_{C=N}$, NH_2 def 1610, 1590, υ_{C-O} 1130, 1100, 1070.
H-NMR ($CDCl_3$): δ 8.45 (1H,s,5-H), 8.1-6.6 (25H,m,arom ring,4'-H,2'-H or 3'-H), 6.35 (1H,s,1'-H), 6.0 (2H,bs,NH_2), 4.6 (1H,bs,2'H or 3'H), 3.7 (6H,s,2xOCH_3), 3.55 (2H,m,5'-CH_2).

1-(5'-0-dimethoxytrityl-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (10)

Cpd. 7 (24.5 g, 0.045 mol) was dissolved in 100 mL dry pyridine and to it 2.4 g of N,N-dimethylaminopyridine and (27.16 mL, 0.224 mol), isobutyric anhydride were added. The mixture was stirred at room temperature for 24 h. It was poured onto 300 mL ice and extracted (2 x 400 mL) with chloroform. The combined organic extracts were washed with 1 M $NaHCO_3$ (2 x 400 mL), water (400 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oil was purified on a silica column with $CHCl_3$:MeOH (40:1) as eluent. This gave 18.0 g (58.5%) of product as a white solid.
IR cm^{-1} (nujol mull): υ_{NH} 3460, 3340, $\upsilon_{C=O}$ 1740, 1690, $\upsilon_{C=O,C=N}$ 1600, 1580.
^{1}H-NMR ($CDCl_3$): δ 8.45 (s, 1H, 5-H), 7.7-6.7 (m, 14H, NH + 13 arom), 6.4 (bs, 1H, NH), 6.1 (s + m, 2H, 1H + 2'H or 3'H), 5.7 (t, 1H, 4'H), 4.4 (m, 1H, 2'H or 3'H), 3.85 (s, 6H, 2 x OCH_3), 3.5 (bs, 2H, 5'CH_2), 2.6 (m, 2H, isobutryl CH), 1.25 (2s, 12H, 4 x CH_3).

1-(5'-0-dimethoxytrityl-2',3'-bis-0-acetate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (11)

30.0 g, 0.05489 mol of 1-[5'-0-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide was dissolved in anhydrous methylene chloride (450 mL) and 12.0 g, 0.10978 mol of dry triethylamine was added. After 20 min, acetic anhydride (18.0 g, 0.176315 mol) was added dropwise and the solution was stirred overnight at room temperature. It was washed with 5% $NaHCO_3$ (2 x 500 mL), H_20 (500 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. This gave 32.0 g (96.1%) of produce as a white solid.

IR cm^{-1} (nujol mull): υ_{NH} 3480, 3340, $\upsilon_{C=O}$ 1755, 1700, $\upsilon_{C=C,C=N}$ 1610, 1590.

^{1}H-NMR (DMSO-d_6): δ 8.926 (s,1H,5-H); 7.766 (s,2H,NH_2); 7.286 (m,9H,arom,J=3.4 Hz); 5.64 (t,1H,3'-H,J-5.6 Hz); 4.325 (apparent q,1H,4'-H); 3.734 (s,6H,2xOCH_3); 3.25 (m,2H,5'-CH_2); 2.104 (s,3H,$OCOCH_3$); 2.049 (s,3H,$OCOCH_3$).

1-(2',3'-bis-0-pivaloate-β-D-ribofuranosyl)-1,2,3-triazole-3-carboxamide (12)

Cpd. 8 (3.0 g, 4.2 mmol) was dissolved in 60 mL 80% acetic acid and the mixture was stirred at room temperature for 1 h. It was neutralized with solid sodium bicarbonate until no more gas evolved, and was diluted with 300 mL water. The water layer was washed (2x600 mL) with Petroleum-ether 40-60° and then extracted 2x300 mL) with methylene chloride. The organic layer was washed with 200 mL water, dried ($MgSO_4$ and the solvent was removed under reduced pressure to give 1.43 g (82.7%) of product as a white solid.

IR cm^{-1} (nujol mull): υ_{NH} 3500, 3380, υ_{OH} 3140, υNH_2def 1640, $\upsilon_{C=O}$ 1740, 1710, 1680, υ_{C-O} 1130, 1160.

H-NMR ($CDCl_3$ DMSO-d_6): δ 8.8 (1H, S, 5-H), 7.55-7.1 (2H 2bs, NH_2), 6.05 (1H, d, 1'-H), 5.9-5.35 (2H, m, 2'-H, 3'-H), 4.3 (1H, m, 4'-H), 3.75 (2H, bs, 5'-CH_2, 3.1 (1H, bs, OH), 1.2 (18H, 2s, 6xCH_3).

1-(2',3'-bis-0-benzoate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (13)

Cpd. 9 (5.0 g, 6.62 mmol) was dissolved in 80 mL of 80% acetic acid and the mixture was stirred at room temperature for 1 h. It was neutralized with solid sodium

bicarbonate until no more gas evolved, and was diluted with 500 mL water. The white precipitate formed was filtered off and washed thoroughly with petroleum ether (40-60°). It was dissolved in 250 mL methylene chloride, washed with 200 mL water, dried ($MgSO_4$) and the solvent was removed under reduced pressure to give 1.7 g (56.9%) of product as white solid.

IR cm^{-1} (nujol mull): υ_{NH} 3440, 3330, 3180, $\upsilon_{C=O}$ 1730, 1700, $\upsilon_{C=N,\ C=C}$, NH_2 def 1630, 1600 1590, υ_{C-O} 1120, 1100, 1070, 1030.

^{1}H-NMR (DMSO-d_6): δ 9.05 (1H, s, 5-H), 8.3-7.3 (m, 2 x Ph, NH_2), 6.65 (1H, d, 1'-H), 6.1 (2H, m, 2'-H, 3'-H), 5.35 (1H, t, 5'-OH), 4.65 (1H, m, 4'-H), 3.85 (2H, m, 5'-CH_2.

1-(2',3'-bis-0-isobutyrate-β-D-ribofuranosyl)-1,2,3-triazole-3-carboxamide (14)

Cpd. 1o (13.0 g, 0.019 mol) was dissolved in 150 mL 80% acetic acid and the mixture was stirred at room temperature for 1 hr. It was neutralized with solid sodium bicarbonate until no more gas evolved, and was diluted with 1000 mL water. The aqueous layer was washed (2 x 500 mL) with ether and then extracted with chloroform (2 x 500 mL). The organic layer was washed with water (700 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure to give 6.0 g (82.5%) of product as a white solid.

IR cm^{-1} (nujol mull): υ_{OH+NH} 3480, 3380, 3160, $\upsilon_{C=O}$ 1740, 1700, $\upsilon_{C=C,C=N}$ 1670, 1640.

^{1}H-NMR (DMSO-d_6): δ 8.85 (s, 1H, 5-H), 7.5 (bs, 2H, NH_2), 6.15 (d, 1H, 1'H), 5.7 (m, 2H, OH + 2'H or 3'H), 5.15 (t, 1H, 4'H), 4.3 (m, 1H, 2'H or 3'H), 3.8 (bs, 2H, 5'CH_2), 2.6 (m, 2H, isobutyl CH), 1.2 (2s, 4xCH_3).

1-(2',3-bis-0-acetate-β-C-ribofuranosyl)-1,2,4-triazole-3-carboxamide (15)

(32.0 g, 0.05275 mol) of 1-[5'-0-(4,4'-dimethoxytrityl)-2',3'-bis-0-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide was dissolved in 80% acetic acid (100 mL) and the mixture was stirred at room temperature for 30 min. 1.5 L of petroleum-ether:ether (2:1) was added and the solution was stirred for 1 h. The solvent was decanted off and the remaining oily solid extracted with 400 mL CH_2Cl_2, dried ($MgSO_4$) and the solvent removed in vacuo. The resulting solid was washed with 1 L ether. 14.7 g (91.9%) of product was obtained as a white solid.

IR cm^{-1} (nujol mull): υ_{NH+OH} 3560, 3360, 3300, 3180, $\upsilon_{C=O}$ 1750, 1700, $\upsilon_{C=N}$ 1630.
^{1}H-NMR (DMSO-d_6: δ 8.915(s,1H,5-H); 7.932(s,1H,NH); 7.742(s,1H,NH); 6.267(d,1H,1'-H,J= Hz); 5.94(t,1H,2'-H,J=5 Hz); 5.488(t,1H,3'-H,J=5 Hz); 4.602(bs,5'-OH+H_2O); 4.249(apparent q,1H,4'-H,J=4 Hz); 3.66(m,2H,5'-CH_2); 2.103 and 2.079(2s,6H,2xCH_3).

1-[5'-(3-carbonylpyridine)-2',3'-bis-0-pivaloate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (16)

Cpd. 12 (1.0 g, 2.4 mmol) was dissolved in 30 mL anhydrous pyridine and cooled at 0°C. Nicotinoyl chloride hydrochloride (0.87 g, 4.87 mmol) was added to the solution portionwise and the mixture was stirred overnight at room temperature. It was then poured into 200 mL ice water and extracted with chloroform (2 x 200 mL). The organic layer was washed with $NaHCO_3$ (2 x 200 mL) and water (200 mL). It was dried over sodium sulfate and the solvent was removed under reduced pressure. The resulting oil was dissolved in a minimum amount of dry ether and allowed to stand. A white solid precipitated. This was collected by filtration and washed with a small amount of cold ether. 0.85 g (67.5%) of the desired product was obtained as a white solid.
IR cm^{-1} (nujol mull): υ_{NH} 3430, 3340, 3300, 3220, υ_{C-O} 1740, 1710, 1690, $\upsilon_{C=N}$, NH_2 def 1620, 1600, $\upsilon_{C-)}$ 1150, 1130, 1100.
^{1}H-NMR ($CDCl_3$): δ 9.3(1H,s,pyr), 8.8(1H,df,pyr), 8.4(1H,s,5-H), 8.35(1H,m,pyr), 8.8(1H,df,pyr), 8.4(1H,s,5-H), 8.35(1H,m,pyr), 7.4(1H,m,pyr), 7.1(1H,bs,NH), 6.25 (1H,bs,NH), 6.1(1H,s,1'-H), 5.8(2H,m,2'-H,4'-H), 4.65 (2H,bs,5'-CH_{23}'H), 1.25(18H,s,6xCH_3).

1-[5'-(3-carbonylpyridine)-2',3'-bis-0-benzoate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (17)

Cpd. 13 (1.0 g, 2.22 mmol) was dissolved in 30 mL dry pyridine and the solution was cooled to 0°C. Nicotinoyl chloride hydrochloride (0.79 g, 4.42 mmol) was added to the solvent portionwise and the mixture was stirred at room temperature overnight. It was poured into 200 mL ice and extracted with chloroform (2 x 200 mL). The organic extracts were washed with NaHCO3 (2 x 200 mL), water (200 mL) and dried over sodium sulfate. The solvent was removed in vacuo and the remaining oil was dissolved

in a small amount of ether and evaporated. The resulting white foam was recrystallized from EtOAc/ether/Pet. ether to give 0.7 g (56.8%) of a white solid.
IR cm-1 (nujol mull): υ_{NH}3480, 3370, $\upsilon_{C=O}$1745, 1725, 1690, υ_{C-N}, NH_2 def 1600, 1590, υ_{C-O} 1120, 1090, 1070.
^{1}H-NMR (DMSO-d_6): δ 9.15 (1H,s,pyr), 9.0(1H,s,5-H), 8.85(1H,d,pyr), 8.45(1H,d,pyr), 8.25-7.1(m,2xPh,pyrH,2xNH), 6.75(1H,s,1'-H), 6.15(2H,bs,2'-H,4'-H), 4.8(m,3'-H,5'-CH_2).

1-[5'-(3-carbonylpyridine)-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl]-1,2,3-triazole-3-carboxamide (18)

Cpd. 14 (5.0 g, 0.013 mol) was dissolved in 100 mL dry pyridine and the solution was cooled to 0°C. (5.9 g, 0.026 mol) nicotinic anhydride was added to it and the mixture was stirred overnight at room temperature. It was poured onto 200 mL ice and extracted with chloroform (2 x 300 mL). The combined organic extracts were washed with 1M NaHCO3 (2 x 300 mL), water (300 mL) and dried (MgSO4). The solvent was removed in vacuo and the resulting oil was dissolved in a small amount of ether and evaporated. 5.5 g (86.4%) of product was obtained as a white foam.
IR cm-1 (nujol mull): υ_{NH} 3460, 3340, 3180, $\upsilon_{C=O}$ 1740-1690, $\upsilon_{C=C,C=N}$ 1600.
^{1}H-NMR ($CDCl_3$): δ 9.3(s,1H,Pyr C2), 8.85(d,1H,Pyr C4), 8.55(s,1H,5-H), 8.45(d,1H,Pyr C_6), 7.5(m,1H,Pyr C_5, 7.35(bs,1H,NH), 6.8(bs,1H,NH), 6.2(d,1H,1'H), 5.9(m,2H,2'H or 3'H+4'H), 4.8(bs,3H,5'CH_2+2'H or 3'H), 2.65(m,2H,isobutyl CH), 1.3(2s,4xCH_3).

1-[5'-(3-carbonylpyridine)-2',3'-bis-0-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (19)

(5.0 g, 0.01649 mol) of 1-(2',3'-bis-0-acetate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide was stirred with 300 mL anhydrous methylene chloride and (1.66 g, 0.01649 mol) dry triethylamine. Nicontinic anhydride (8.3 g, 0.03637 mol) was added and the mixture stirred at room temperature for 48 h. It was washed with $NaHCO_3$ (2 x 500 mL), H_2O (300 mL), dried ($MgSO_4$) and the solvent was removed in vacuo. The resulting solid was purified on a silica column with chloroform:methanol (10:1) as eluant. This gave 3.8 g (56.4%) of product as white solid.
IR cm-1 (nujol mull): υ_{NH} 3460, 3340, 3280, 3200, $\upsilon_{C=O}$ 1750, 1690, $\upsilon_{C=N,C=C}$ 1600.

^{1}H-NMR (DMSO-d_6): δ 9.099(d,1H,pyr C2,J=1.4 Hx); 8.910(s,1H,5-H); 8.838(dd,1H,pyr C-6,J=3.2 and 1.6 Hz); 8.401(dt,1H,pyr C-4,J=8 + 2 Hz); 7.924(s,1H,NH); 7.788(s,1H,NH); 7.620(dd,1H,pyr C-5,J=4.8 Hz); 6.431(d,1H,1'-H,J=2.4 Hz); 5.763(m,2H,2'-H,3'-H); 4.594(m,3H,4'-H + 5'CH_2); 2.133 and 2.102(2s,6H, 2 x $OCOCH_3$).

1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-bis-0-pivaloate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (20)

Cpd. 16 (0.8 g, 1.55 mmol) was dissolved in 100 mL dry acetone. 2.7 g of methyl iodide were added and the mixture was refluxed overnight. The solvent was removed under reduced pressure and ether was added to the resulting gum. The yellow precipitate was filtered (hygroscopic) and dried in vacuo. This gave 9.9 g (88.2%) of (20).

U.V. λmax (MeOH): 266, 217

IR cm^{-1} (nujol mull): υ_{NH} 3440, 3300, $\upsilon_{C=O}$ 1735, 1685, $\upsilon_{C=N}$, NH_2 def 1640, 1590, υ_{C-O} 1130, 1160.

^{1}H-NMR (DMSO-d_6): δ 9.55 (1H,s,pyr), 9.3(1H,d,pyr), 9.1(1H,d,pyr), 9.0(1H,s,5-H), 8.3(1H,m,pyr), 7.8-7.7(2H,2xbs,NH_2), 6.45(1H,s,1'-H), 5.8(2H,bs,4'-H,2'-H), 4.7(3H,bs,5'-CH_2,3'-H), 4.5(3H,s,N-CH_3), 1.2(s,6xCH_3).

1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-bis-0-benzoate-β-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (21)

Cpd. 17 (0.7 g, 1.35 mmol) was dissolved in 60 mL dry acetone. 2.4 g of methyl iodide were added and the mixture was refluxed overnight. The solvent was removed under reduced pressure and to the resulting gum a minimum amount of ethyl acetate was added. The resulting yellow solid was filtered and washed with a small amount of cold ethyl acetate. This gave 0.75 g (77.9%) of the desired salt.

U.V. λ_{max} (MeOH): 266, 223, 209

IR cm^{-1} (nujol mull): υ_{NH} 3440, 3300, 3280, 3180, $\upsilon_{C=O}$ 1750, 1730, 1690, $\upsilon_{C=C,C=N}$ NH_2 def 1650, 1600, υ_{C-O} 1130, 1100.

^{1}H-NMR (DMSO-d_6) δ 9.65(1H,s,pyr), 9.35(1H,d,pyr), 9.1(1H,d,pyr), 9.0(1H,s,5-H),

8.35(1H,m,pyr), 8.15-7.3(12h,m,2xPh,NH_2), 6.8(1H,s,1'-H), 6.2(2H,bs,2'-H,4'-H), 5.2-4.7(3H,m,5'-CH_2,3'-H), 4.55(3H,s,N-CH_3).

1-(5'-[1-methyl-3-carbonylpyridinium]-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide iodide (22)

Cpd. 18 (5.0 g, 0.0102 mol) was dissolved in 100 mL dry acetone. 5.0 g methyl iodide were added and the mixture was refluxed for 48 h. The solvent was removed under reduced pressure and the resulting yellow gum was dissolved in 100 mL water and extracted with chloroform (2 x 50 mL). The aqueous layer was freeze dried to give 5.6 g (86.9%) of product as a yellow solid.

UV λmax (MeOH): 265.8, 217.8.

IR cm-1 (nujol mull): υ_{NH} 3460, υ_{C-O} 1740, 1690, $\upsilon_{C=C,C=N}$ 1650, 1600.

^{1}H-NMR ($CDCl_3$/DMSO-d_6 δ 9.65(s,1H,Pyr C_2), 9.5(d,1H,Pyr C_6, 9.15(d,1H,Pyr C_6), 8.85(s,1H,5-H), 8.35(m,1H,Pyr C_5, 7.45(bd,2H,NH_2), 6.35(d,1H,1'H), 5.85(m,2H,4'H+2'H or 3'H), 4.65(m+s,6H,N-CH_3+5'CH_2+2'H or 3'H), 2.6(m,2H,isobutyl CH), 1.2(2s,12H,4xCH_3).

1-[5'-(1-methyl-3-carbonyl pyridinium)-2',3'-bis-O-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (23)

To 2.5 g (0.006123 mol) of 1-[5'-(3-carbonylpyridine)-2',3'-bis-O-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide in 100 mL of anhydrous acetonitrile, 2.5 g of methyl iodide was added and the mixture refluxed overnight. The solvent was removed in vacuo, and the resulting yellow solid was washed with ether and recrystallized from a mixture of acetone-ether. This gave 2.6 g (77.2%) of the product as a yellow solid.

IR cm^{-1} (nujol mull): υ_{NH}(3440,3320,3260,3180), $\upsilon_{C=O}$(1740,1690), $\upsilon_{C=N,C=C}$(1640,1660).

UV λ_{max} (MeOH): 265.5, 216.5.

^{1}H-NMR (DMSO-d_6): δ 9.545(s,1H,pyr C_2); 9.244(d,1H,pyr C-6, J=6 Hz); 9.093(d,1H,pyr C-4,J=8.2 Hz); 8.908(s,1H,5-H); 8.299(dd,1H,pyr C-5,J=6 and 2 Hz); 7.965(s,1H,NH); 7.735(s,1H,NH); 6.413(d,1H,1'-H,J=2.6 Hz); 5.753(m,2H,2'-H + 3'-H); 4.61(m,3H,4'-H, + 5'-CH_2); 4.453(s,3H,N-CH_3); 2.123 and 2.103(2s,6H,2xOCOCH_3).

1-[5'-(N-methyl-3-carbonyl-1,4-dihydropyridine)-2',3'-bis-O-pivaloate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (24, AVS 5056)

To a stirred, degassed,ice-cold deionized water (250 mL) and chloroform (50 mL) biphasic solution of Cpd. 20 (0.6 g, 0.91 mmol), a mixture of sodium bicarbonate (0.46 g, 5.5 mmol) and sodium dithionite (0.87 g, 5.0 mmol) was added portionwise. The reaction was maintained at 0oC and under argon. After 1 h 15 min the organic layer was separated and the aqueous layer was extracted with 2 x 100 mL cold chloroform. The combined chloroform extracts were washed with 2 x 100 mL cold deionised water, dried (Na_2SO_4) and the solvent was removed in vacuo. 0.46 (95.8%) of product was obtained as a yellow oil.

UV λ_{max} (MeOH): 362,210.

IR (nujol mull) cm^{-1}: υ_{NH} 3480, 3340, 3200, 3110, υ_{CH} 3010, 2980, 2940, 2880, 2820, $\upsilon_{C=O}$ 1740, 1690, $\upsilon_{C=N}$, NH_2 def 1600, υ_{C-O} 1180, 1160, 1130, 1070, 1030.

^{1}H-NMR ($CDCl_3$): δ 8.55(s,1H,5-H), 7.45(s,1H,pyr C_2), 7.3(bs,1H,NH), 7.15(s,1H,1'-H), 6.8(bs,1H,NH), 6.15(d,1H,pyr C_6), 6.0-5.6(m,3H,3'-H,2'-H,4'-H), 4.9(m,1H,pyr,C_5), 4.6(m,2H,5'-CH_2), 3.15(bs,2H,pyr C_4), 3.05(s,3H,N-CH_3), 1.25(s,6 x CH_3).

1-[5'-(N-methyl-3-carbonyl-1,4-dihydropyridine)-2',3'-bis-O-benzoate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (25, AVS 5057)

To stirred, degassed ice-cold deionised water (800 mL) and chloroform (200 mL) system of Cpd. 21 (0.5 g, 0.72 mmol) a mixture of sodium bicarbonate (0.72 g, 8.58 mmol) and sodium dithionite (1.36 g, 7.86 mmol) was added. The reaction was maintained at 0°C and under argon. After 1 h 50 min the organic layer was separated and the aqueous layer extracted with 2 x 250 mL cold chloroform. The combined organic extracts were washed with 2 x 250 mL cold deionised water, dried (sodium sulfate) and removed under reduced pressure to give 0.34 g (83.1%) of the product as a yellow solid.

UV λ_{max} (MeOH): 361, 260, 228, 209.

IR (nujol mull) cm^{-1}: υ_{NH} 3460, 3360, 3180, $\upsilon_{C=O}$ 1730, 1685, $\upsilon_{C=C,\ C=N}$, NH_2 def 1650, 1590, υC=O 1180, 1130, 1100, 1070.

^{1}H-NMR ($CDCl_3$): δ 8.45(s,1H,5-H), 8.15-7.2(m,13H,2 x Ph,pyridine C_2, NH_2),

7.1(s,1H,1'-H), 6.75-5.8(m,2'-H,3'-H,4'-H), 5.6(d,1H,pyridine C_6), 5.0-4.3(m,pyridine C_5,5'-CH_2), 3.1(bs,2H,pyridine C_4), 2.9(s,3H,N-CH_3).

1-[5'-(N-methyl-3-carbonyl-1,4-dihydropyridine)-2',3'-bis-O-isobutyrate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (26,AVS 5054)

5.5 g of quaternary compound (22) was dissolved in 500 mL ice-cold deionized water and extracted with chloroform (2 x 400 mL). The aqueous layer was degassed and cooled to 0°C. A mixture of sodium bicarbonate (6.0 g, 0.071 mol) and sodium dithionite (11.6 g, 0.067 mol) was added portionwise to the stirred solution. After 1 h 40 min it was extracted with ice-cold ethyl acetate (600 mL). The organic layer was washed with ice-cold water (500 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure to give 3.1 g (77.5%) of product as a yellow solid.
UV λmax (MeOH): 360, 213.5.
IR cm^{-1} (nujol mull): υ_{NH} 3460, 3340, 3100, $\upsilon_{C=O}$ 1750, 1690, $\upsilon_{C=C,C=N}$ 1660, 1590.
^{1}H-NMR ($CDCl_3$): δ 8.4(s,1H,5-H), 7.3-7.2(s + bs, 2H, NH + -yr C_2), 6.08(d,1H,1'H), 5.9-5.7(t + bs,2H,4'h + NH), 5.68(d,1H,pyr C_6), 4.88(dt,1H,pyr C_5), 4.66-4.44(d + m,3H,2'H or 3'H + 5'CH_2), 4.16(d,1H,2'H or 3'H), 3.17(m,2H,pyr C_4), 3.01(s,3H,NCH_3), 2.7(m,2H,isobutyl CH), 1.4(m,12H,4 x CH_3).

1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine)-2',3'-bis-O-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (27, AVS 5581)

To a stirred, degassed, ice-cold deionized water (50 mL) and methylene chloride (50 mL) solution containing (0.5 g, 0.00090987 mol) of 1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-bis-O-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide, a mixture of sodium dithionite (0.63 g, 0.003635 mol) and sodium bicarbonate (0.31 g, 0.003635 mol) was added. The reaction was maintained at 0oC and under argon. After 2.5 h, the aqueous layer was extracted with ice-cold ethyl acetate and the combined organic extracts were washed with ice-cold water, dried ($MgSO_4$) and the solvent was removed in vacuo. This gave 0.29 g (75.2%) of product as a yellow solid.
IR cm^{-1} (nujol mull): υ_{NH}(3480,3340,3260,3200), $\upsilon_{C=O}$(1750,1680), $\upsilon_{C=N,C=C}$(1660,1600).
UV λ_{max} (MeOH): 346,225.

^{1}H-NMR (DMSO-d_6): δ 8.386(s,1H5-H), 7.132(bs,1H,NH), 7.012(s,1H,pyr C-2), 6.35(bs,1H,NH), 6.066(d,1H,1'-H,J=4.4 Hz), 5.8210(t,1H,3'-H or 2'-H,J=4.6 Hz), 5.62(dd,H,pyr C-6,J=1.6 + 6.6 Hz), 5.569(t,1H,2'H or 3'H,J=4.4 Hz), 4.792(dt,1H,pyr C-5,J=8 + 4 Hz), 4.517(m,2H,5'-CH_2), 4.277(dt,1H,4'-H,J=8 + 4 Hz), 3.058(bs,2H,pyr C-4), 2,946(s,3H,N-CH_3), 2.132 and 2.111(2s,6H,2 x $OCOCH_3$).

1-[5'-(3-carbonylpyridine)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (28)

5.0 g of 1-[5'-(3-carbonylpyridine)-2',3'-O-isopropylidene-β-D-ribofuranoxyl-1,2,4-triazole-3-carboxamide (6) was dissolved in 100 mL of 88% formic acid and the mixture was stirred at room temperature for 10 h and then at 0°C overnight. Solvent was evaporated in vacuo at 30°C and traces of formic acid were removed by repeated addition of water and evaporation in vacuo. The residue was stirred with methylene chloride (250 mL) for 10-15 min. The solid was filtered and washed with CH_2Cl_2. This gave 4.3 g (95.6%) of the product as a white solid.

IR cm^{-1} (nujol mull): υ_{NH+OH}(3480,3420,3300,3260,3180), $\upsilon_{C=O}$(1760,1700), $\upsilon_{C=C,C=N}$(1670,1600).

^{1}H-NMR (DMSO-d_6): δ9.080(s,1H,pyr C-2); 8.877(s,1H,5-H); 8.825(d,1H,pyr C-6,J=3.8 Hz); 8.368(d,1H,pyr C-4,J=8 Hz); 7.875(s,1H,NH); 7.694(s,1H,NH); 7.628(dd,1H,pyr C-5, J=4.8 + 7.8 Hz); 5.98(d,1H,1'H,J=2.4 Hz); 5.76(d,1H2'OH,J=3.6 Hz); 5.483(d,1H,3'OH,J=6.6 Hz); 4.438(m,5H,5'-CH_2,2'-H,3'-H,4'-H).

1-[5'(1-methyl-3-carbonylpyridinum)-β-D-ribofuranosyl]1,2,4-triazole-3-carboxamide iodide (29)

To 2.3 g of 1-[5'-(3-carbonylpyridine)-β-D-ribofuranoxyl]-1,2,4-triazole-3-carboxamide in 200 mL of anhydrous acetonitrile, 2.3 g methyl iodide was added and the mixture was refluxed overnight. The solvent was removed in vacuo and the resulting solid was washed with ether, followed by methylene chloride. The solid was dissolved in acetone (1000 mL) and triturated with ether. This gave 2.8 g (85.7%) of pale yellow solid.

1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine)-β-D-ribofuranosyl]1,2,4-triazole-3-carboxamide (30)

To a stirred, degassed, ice-cold deionized water (50 mL) solution of 0.8 g of 1-[5'-(1-methyl-3-carbonylpyridinium)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide, a mixture of sodium bicarbonate (8.0 g) and sodium dithionite (11.2 g) was added. The reaction was maintained at 0°C and under argon. After two hours, the solution was freeze dried. The resulting solid was extracted with acetone. The solvent was removed in vacuo and the yellow solid was redissolved in a small amount of acetone and triturated with methylene chloride. The precipitated product was filtered and washed with ether. This gave 0.3 g (52.8%) of yellow solid.
IR cm^{-1} (nujol mull): $\upsilon_{NH11+OH}$(3540-3200), $\upsilon_{C=O}$(1680) υC=C,C=N(1630,1620,1600)
UV λ_{max} (MeOH): 218.5,360.

1-(2',3'-O-cyclopentylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (31)

20.56 g (0.244 mol) of cyclopentanone was added dropwise to an ice cold stirred solution of mesitylene sulphonic acid (0.36 g, 0.0015 mol) in anhydrous DMF (50 mL) and triethylorthoformate (4.82 g, 0.0325 mol). On completion of addition, the mixture was stirred at RT for 2 h. Ribavirin (5.0 g, 0.0205 mol) was added to the solution and it was stirred at RT until a clear solution was obtained (overnight). The solution was neutralized with triethylamine and evaporated under reduced pressure to give a pale brown oil. This was dissolved in chloroform and washed with a small amount of water. The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure to give 4.0 g (63%) of product as a white solid.
^{1}H-NMR (DMSO-d_6): δ 8.82(s,1H,5-H), 7.89(s,1H,NH), 7.69(s,1H,NH), 6.24(s,1H,1'-H), 5.15(d,1H,J=6Hz,2'-H), 4.99(t,1H,J=5.4 Hz,5'-OH), 4.87(d,1H,J=6Hz,3'-H), 4.26(t,1H,J=6 Hz,4'-H), 3.46(m,2H,5'-CH2), 1.93+1.63(m,2H+6H, cyclopent)
IR cm^{-1} (nujol mull): υ_{NH+OH} 3380 and 3180; 1660.

1-[5'-(3-carbonylpyridine)-2',3'-O-cyclopentylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (32)

1.0 g (0.0032 mol) of 2',3'-O-cyclopentylidene-β-D-ribofuranosyl-1,2,4-

triarocarboxamide was dissolved in 25 mL anhydrous pyridine. 1.5 g (0.0064 mol) of nicotinic anhydride was added to it and the reaction mixture was stirred at RT for 24 h. It was poured onto 50 mL ice and extracted with methylene chloride (2 x 50 mL). The combined organic extracts were washed with 5% $NaHCO_3$ (2 x 40 mL), 40 mL H_2O, dried ($MgSO_4$) and the solvent was removed v.r.p. to give 1.1 g (82.2%) of product as a white solid.

^{1}H-NMR (DMSO-d_6): δ 9.01(s,1H,pyr 2-H), 8.85(s,1H,5-H), 8.82(dd,1H,pyr 6-H,J=6+2 Hz), 8.23(dt,1H,pyr 4-H,J=8+2 Hz), 7.88(s,1H,NH), 7.70(s,1H,NH), 8.06(dd,1H,pyr 5-H,J=8+5 Hz), 6.39(s,1H,1'H), 5.23(d,1H,3'-H,J=6 Hz), 5.14(dd,1H,2'-H,J=8+2 Hz), 4.66(apparent g,111,4'-H), 4.48(m,2H,5'-CH_2), 1.89+1.70(m,4H+4H,cyclopentane).

1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-O-cyclopentylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide (33)

To 1.0 g (0.0024 mol) of 1-[5'-(3-carbonylpyridine)-2',3'-O-cyclopentylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide in 75 mL of anhydrous acetonitrile, 1.1 g methyl iodide was added and the solution was stirred at RT for 5 days. The solvent was removed under vacuo and the resulting solid was washed with methylene chloride, and then triturated with acetone ether. The precipitated product was filtered and washed with ether to give 1.1 g (82%) of product as a yellow solid.

^{1}1H-NMR (DMSO-d_6): δ 9.49(s,1H,pyr 2-H), 9.19(d,1H,pyr 6-H,J=4.81 Hz), 8.94(d,1H,pyr 4-H,J=5 Hz), 8.86(s,1H,5-H), 8.25(dd,1H,pyr 5-H,J=5+2 Hz), 7.85(s,1H,NH), 7.63(s,1H,NH), 6.36(s,1H,1'-H), 5.25(s,1H,3'-H), 5.18(s,1H,2'-H), 4.54(m,3H,4'H+5'-CH2), 4.44(s,3H,pyr N-CH_3), 1.96+1.6(m,4H+4H,cyclopentane).

UV λ_{max} (MeOH): 216,266.

IR cm^{-1} (nujol mull): υ_{NH} 3440 and 3210,3180, $\upsilon_{C=O}$ 1730, $\upsilon_{C=N,C=C}$ 1680, 1640.

1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine-2',3'-O-cyclopentylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (34)

To a stirred, degassed, ice-cold deionized water (50 mL) and ethyl acetate (50 mL) solution containing 0.7 g (0.00117 mol) of 1-[5'-(1-methyl-3-carbonylpyridinium)-2',3'-O-cyclopentylidene-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide iodide, a mixture

of sodium bicarbonate (0.6 g, 0.007 mol) and sodium dithionite (0.8 g, 0.005 mol) was added. The reaction was maintained at 0°C and under argon. After 1 h 45 min the organic layer was separated. The aqueous layer was extracted with ice-cold ethyl acetate and the combined organic extracts were washed with ice-cold, dried ($MgSO_4$) and the solvent removed under reduced pressure. This gave 0.4 g (79%) of product as a yellow solid.

^{1}H-NMR ($CDCl_3$): δ 8.34(s,1H,5-H), 7.20(s,1H,NH), 6.76(s,1H,2-H), 6.43(s,1H,NH), 6.06(s,1H,1'-H), 5.60(dd,1H,pyr 6-H,J=8+2 Hz), 5.28(d,1H,2'-H,J=6 Hz), 4.85(dd,1H,3'-H,J=6+2 Hz), 4.76(dt,1H,pyr 5-H,J=8+4 Hz), 4.65(dt,1H,4'-H,J=8+3 Hz), 4.30+4.20(dd,2H,5'-CH_2,J=22+5 Hz), 2.04+1.75(m,2H+6H,cyclopentane).

UV λ_{max} (MeOH): 211,359.

IR cm^{-1} (nujol mull): υ_{NH} 3460,3340 and 3200, $\upsilon_{C=O}$ 1720, $\upsilon_{C=N,C=C}$ 1650,1580.

1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (36)

Ribavirin (1.0 g, 0.004095 mol) was dissolved in anhydrous pyridine (30 mL) and the solution was cooled to 0°C. 4,4-Dimethylaminopyridine (0.15 g, 0.0012 mol) and benzoic anhydride (3.1 g, 0.0135 mol) were added to it and the mixture was stirred at 80°C overnight. The pyridine was removed in vacuo, the resulting oil dissolved in CH_2Cl_2 and washed with 5% $NaHCO_3$, 5% HCl and H_2O. The methylene chloride solution was dried over $MgSO_4$ and the solvent was removed in vacuo. The crude white solid was purified on a silica column with CH_2Cl_2:MeOH:Pet. ether, 95:5:20 as eluent. The product was obtained as a white solid in 70% yield.

IR (nujol mull): υ_{NH} 3460,3360, $\upsilon_{C=O}$ 1725,1690, $\upsilon_{C=N}$ 1655, $\upsilon_{C=C}$ 1600, υ_{C-O} 1130-1030.

^{1}H-NMR ($CDCl_3$): δ 8.6(s,1H,triazole), 8.25-7.25(m,15H,3 x C_6H_5), 6.35(d,1H,1'H), 6.15(m,4H,2'H,3'H, NH_2), 4.75(br s + t,3H,5'H,4'H).

1-[3,5-O-(tetraisopropyldisilox-1,3-diyl)-β-D-ribofuranosyl]1,2,4-triazole-3-carboxamide (37)

Ribavirin (10.0 g, 40.95 mmol) was made anhydrous by repeated coevaporation with pyridine. Subsequently, it was dissolved in 300 mL dry pyridine and the solution was cooled to 0°C. 1,1,3,3-dichlorotetraisopropyldisiloxane (13.4 mL) was added to the

reaction vehicle in which atmosphere moisture was excluded and the reaction mixture was stirred at ambient temperature for 24 h. It was then quenched under ice-cooling with a solution of 5% ammonium bicarbonate (500 mL) and extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O, dried ($MgSO_4$), and the solvent was removed under reduced pressure. The clear oil obtained was purified on a silica column by using a step gradient of CH_3OH in $CHCl_3$ (0.5% to 5%). 13.2 g (66.2%) of the product was obtained as a white solid.

IR cm^{-1} (nujol mull): υ_{NH+OH} 3480,3300,3260,3180, υ_{CH} 3120, $\upsilon_{C=O}$ 1700, $\upsilon_{C=N,\ NH2}$ def 1600.

^{1}H-NMR ($CDCl_3$): δ 8.55(s,1H,5-H), 7.15(bs,1H,NH), 6.65(bs,1H,NH), 6.00(s,1H,1'-H), 4.9-4.1(m+s,5H,3'-H,4'-H,2'-H,5'-CH_2), 2.25(m,1H,OH), 1.1(s,28H,8 x CH_3,4 x CH).

Attempted preparation of 1-[3',5'-O-(tetraisopropydisilox-1,3-diyl)-2'-(3-carbonylpyridine)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (38)

Cpd. (37) (1.45 g, 3.0 mmol) was dissolved in 15 mL dry pyridine and cooled to 0°C. 1.59 g (8.9 mmol) of nicotinoyl chloride hydrochloride was added and the mixture stirred at ambient temperature for 24 h. It was poured onto 100 mL ice and extracted (2 x 100 mL) with $CHCl_3$. The combined organic extracts were washed with 2 x 100 mL 5% $NaHCO_3$, 100 mL H_2O, dried ($MgSO_4$) and the solvent was removed *in vacuo*. The resulting oil was purified on a silica column with $CHCl_3$:MeOH (40:1) as eluent. This gave 1.0 g of product as a waxy white solid.

IR cm^{-1} (nujol mull): υ_{C-H} unsat 3140, $\upsilon_{C=N}$ 2260, $\upsilon_{C=O}$ 1740, $\upsilon_{C=N}$ 1595, $\upsilon_{C=O}$ 1170,1130,1090,1050.

^{1}H-NMR ($CDCl_3$): δ 9.2(s,1H,pyr), 8.75(d,1H,pyr), 8.5(s,1H,5-H), 8.25(d,1H,pyr), 7.35(dd,1H,pyr), 6.15(s-1'-H,1H), 5.8 (d,1H,3'-H), 4.95(dd,1H,2'-H), 4.3(bs,1H,4' H), 4.05(bs,2H,5'-CH_2), 1.00(m,28H,4 x isopropyl).

1-[3',5'-O-(tetraisopropyldisilox-1,3-diyl)-2'-(3-carbonylpyridine)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (39)

Cpd. (37) (1.0 g, 0.002 mol) was dissolved in 20 mL dry pyridine and cooled to 0°C. (0.94 g, 0.0041 mol) nicotinic anhydride was added and the mixture stirred at room temperature for 24 h. It was poured onto 200 mL ice and extracted (2 x 200 mL) with

chloroform. The combined organic extracts were washed with1M $NaHCO_3$ (200 mL), water (200 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oil was stirred with petroleum-ether (40-60°) to give the product as a white solid (1.1 g, 90.5%).

IR cm^{-1} (nujol mull): υ_{NH} 3460,3340, $\upsilon_{C=O}$ 1745,1700,1690, $\upsilon_{C=C,C=N}$ 1600,1580.

^{1}H-NMR ($CDCl_3$): δ 9.45(s,1H,pyr), 8.95(d,1H,pyr), 8.65(s,1H,5-H), 8.5(d,1H,pyr), 7.55(m,1H,pyr), 7.2-7.0(2 x bs,2H,NH_2), 6.25(s,1H), 6.0(d,1H), 4.95(m,1H), 4.4(m,1H), 4.2(bs,2H), 1.0(m,28H).

Reaction of 1-(5'-O-dimethoxytrityl-β-D-ribofuranosyl)-1,2,4-triazole-3-N-carboxamide with benzoyl chloride (40, 41 and 42)

Ribavirin-DMT (7) (0.55 g, 0.001 mol) was coevaporated with dry pyridine (3 x 3 mL) and then dissolved into 5 mL of dry pyridine. The solution was cooled in an ice-cold bath and then benzoyl chloride (0.16 g, 0.0011 mol) in 5 mL of anhydrous pyridine was added dropwise over a period of 30 minutes. The reaction mixture was left overnight stirring and then quenched with 5% solution of $NaHCO_3$ (100 mL) and extracted with methylene chloride (4 x 10 mL) and dried with $MgSO_4$. After solvent removal the crude material (0.94 g) was purified by column chromatography on silica gel (100-200 mesh) using 2% MeOH in $CHCl_3$ as an eluent.

As determined by spectral methods the first isolated product (130 mg) was 2',3'-dibenzoyl derivative (40). Then the mixture of monobenzoylated products (200 mg) and pure one monobenzoylated compound. 2',3'-Dibenzoyl ester (40) exists as a monohydrate. For $C_{43}H_{38}N_4O_9$ calculated: C, 68.42; H, 5.07; N, 7.42. Found for $C_{43}H_{38}N_4O_9 \times H_2O$: C, 66.86(66.82), H, 4.89(4.95); N, 7.21(7.25). ^{1}H-NMR ($CDCl_3$) (200 MHz, FT mode) δ(ppm): 8.43(1H,s), 7.96-7.91(4H,m), 7.60-7.15(17H,m), 6.82(2H,s), 6.78(2H,s), 6.72(1H,m), 6.40-6.30(2H,m), 6.10-5.95(2H,m), 4.64-4.62(1H,m), 3.75(6H,s), 3.60-3.50(2H,m). ^{13}C-NMR ($CDCl_3$)(50 MHz, FT mode) δ(ppm): 16.51(CO), 160.3($CONH_2$), 158.6, 157.3. 144.5, 144.2, 135.25, 135.2, 133.7, 133.5, 130.1, 129.75, 129.7, 128.4, 128.1, 127.9, 127.0, 113.2, 90.2, 87.0, 83.05, 74.8, 72.1, 63.0, 55.1.

Repetition of the above reaction with shorter reaction time (3 hrs) led to the

predominant mixture of both monobenzoylated products with a small amount of bis benzoylated product. These two isomers were separated by column chromatography on silica gel using ethyl acetate as an eluent. The proton and carbon spectra of individual isomers were monitored in deuterated chloroform and in DMSO. Use of DMSO as a solvent gives slightly better resolution than chloroform, however, upon standing in DMSO the samples undergo slow isomerization.

Both the 2'- and 3'-isomers were obtained as white solids. Close analysis of proton-proton correlation spectra for both products allowed for determination that the less polar isomer is the desired 2'-benzoyl (41) and the more polar one corresponds to the 3'-benzoyl ester (42).

(41) ^{13}C-NMR (DMSO)(75 MHz, FT mode) δ(ppm): 164.9, 160.2, 158.0, 157.9, 157.7, 145.8, 144.7, 135.5, 133.7, 129.7, 129.65, 129.6, 128.8, 128.7, 127.8, 127.7, 126.6, 113.1, 88.7, 85.4, 82.9, 76.4, 68.9, 63.1, 55.0.

(42) ^{13}C-NMR (DMSO)(75 MHz, FT mode) δ(ppm): 165.0, 160.3, 158.1, 158.07, 157.8, 146.1, 144.7, 135.5, 135.3, 133.6, 129.8, 129.7, 129.5, 129.4, 128.7, 127.85, 127.7, 126.7, 113.2, 91.2, 85.8, 81.0, 73.5, 72.4, 63.2, 55.0.

Attempted Synthesis of 2'-t-butyldimethylsilyl (TBDMS) derivative (45)

5'-Dimethoxytrityl ribavirin (2.0 g, 3.59 mmol) was dissolved in 50 mL of dry tetrahydrofuran. To this solution was added dry pyridine (1.1 mL, 13 mmol) followed by $AgNO_3$ (0.73 g, 4.3 mmol) which had been finely ground with a mortar and pestle. After the $AgNO_3$ had dissolved (~ 15 min), t-butyldimethylsilyl chloride (0.7 g, 4.67 mmol) was added. There was noted the immediate formation of a white precipitate (AgCl) and the reaction was stirred overnight at room temperature. The reaction was filtered and the filtrate was concentrated in vacuum to give a residue which was taken up into CH_2Cl_2. This solution was washed twice with 0.5 M $NaHCO_3$. The organic layer was separated and dried over $Na2SO_4$. The dried solution was filtered and the filtrate was concentrated in vacuum to an oil. Tlc analysis of this product showed it to be a mixture of three products, which were shown by 1H-NMR analysis to be the 2'-, thte 3'-, and the 2', 3'-bis silylated derivatives of 5'-dimethoxytrityl ribavirin. Tlc analysis on silica (layer = 200 μ) with ethyl acetate as eluent showed two major spots. One spot,

having an R_f of 0.50, was determined to be the 2'-silylated derivative, and another, having an R_f of 0.40 was determined to be the 3'-silyl compound. A third spot, Rf equal to 0.62, was determined to be the 2', 3'-bis silyl compound. The mixture was separated by chromatography from 250 g of silica gel, using ethyl acetate/hexane mixtures. The ^{1}H-NMR spectrum of the fraction which eluted secondly shows ($CDCl_3$) δ(ppm) 8.38(s,1,5-H), 5.84(d,1,J=6Hz,1'-H), 3.74(s,6,OCH_3), 0.87(s,9,-$SiCCH_3$). The Rf of this compound in ethyl acetate is also consistent with the results of Hakimelahi[41] who showed that the 2'-silyl derivatives were generally less polar than the 3'-derivatives. The NMR spectrum of the fraction which eluted thirdly shows ($CDCl_3$) δ(ppm) 8.52(s,1,5-H), 6.00(d,1,J=4Hz,1'-H), 3.76(s,6,OCH_3), 0.85(s,9,-$SiCCH_3$).

Reaction of 5'-dimethoxytrityl ribavirin with nicotinic anhydride (46)

To a solution of 5'-dimethoxytrityl ribavirin (0.65 g, 1.17 mmol) in 40 mL of pyridine was added nicotinic anhydride (0.80 g, 3.5 mmol). The reaction was stirred at room temperature overnight. The appearance of product was noted by TLC analysis. At intervals, the reaction was analyzed by TLC (SiO_2, ethyl acetate, methanol, triethylamine; 4:1:trace). At 45 min, the spot corresponding to the product (R_f = 0.33) was barely detectable. At 3 hrs the spot was nearly equal the intensity of the spot corresponding to the starting material (R_f = 0.41). After stirring overnight, the reaction appeared to be ~95% complete. The reaction mixture was concentrated in vacuo to a solid which was triturated with 30 mL of ethyl acetate for 30 mins. The resulting suspension was filtered. The filtrate was concentrated in vacuo to another residue which was again triturated with ethyl acetate, filtered, and concentrated. The residue from this third concentration was chromatographed from 100 g, of SiO_2, using ethyl acetate as eluent. The product partially decomposed on the column (both trityl and nicotinoyl groups hydrolysed) and the eluate containing the desired compound was concentrated and streaked onto a preparative TLC plate (SiO_2, 2mm). The plate was developed with n-butanol, acetic acid, and water; 4:1:1. the product, with the loss of the dimethoxytrityl group, was removed from the plate but was contaminated with ribavirin. This product, presumably 2'-nicotinoyl ribavirin, has a R_f of 0.24, and stains blue (not orange) with the orcinol spray reagent.

Reaction of 5'-dimethoxytrityl ribavirin with Benzoic Anhydride (47)

To a solution of 5'-dimethoxytrityl ribavirin (0.53 g, 0.95 mmol) in 25 mL of dry pyridine was added benzoic anhydride (0.65 g, 2.86 mmol). The reaction was stirred at room temperature overnight. TLC analysis of the reaction mixture showed that a large amount of starting material remained and that the reaction gave two products, in equal amounts. The reaction was not analyzed further.

Reaction of 5'-dimethoxytrityl ribavirin with Anisic Anhydride (48)

To a solution of 5'-dimethoxytrityl ribavirin (0.69 g, 1.24 mmol) in 20 mL of dry pyridine was added anisic anhydride (1.06 g, 3.72 mmol). The reaction was stirred at room temperature for 2 days. TLC analysis showed the presence of a large amount of starting material and primarily a single product. At this point, DMAP (25 mg, catalytic amount) was added and the reaction was stirred at room temperature another day. TLC analysis at the end of this period showed complete reaction (starting material was not detectable) and a single product. The reaction was concentrated in vacuo but not analyzed further.

1-(5'-9-dimethoxytrityl-3'-benzoyl-2'-nicotinoyl-β-D-ribofuranosyl)-1,2,4-triazole-3-N-carboxamide (49)

The 3'-0-benzoyl ester (42) (0.48 g, 0.74 mmol) (13) was dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.09 g, 0.88 mmol), catalytic amount of DMAP, and nicotinic anhydride (0.17 g, 0.88 mmol) were added and the solution was stirred over a period of 1 hr. Then the reaction was quenched with 5% $NaHCO_3$ (30 mL) and extracted with methylene chloride (3 x 10 mL). The organic layer was washed with water (20 mL) and dried with magnesium sulfate. The crude product was purified by column chromatography on silica gel using ethyl acetate as an eluent. Yield was 73%.
^{1}H-NMR (DMSO)(300 MHz)(FT mode) δ(ppm): 9.09(1H,s), 8.98-8.82(1H,m), 8.34-8.26(2H,m), 7.92-7.84(4H,m), 7.66-7.18(12H,m), 6.84-6.81(4H,m), 6.74-6.73(1H,m), 6.21-6.19(1H,m), 6.11-6.07(1H,m), 4.70-4.65(1H,m), 3.71(6H,s), 3.43-3.41(2H,m).
^{13}C-NMR (DMSO)(75 MHz)(FT mode) δ(ppm): 164.5, 163.6, 160.15, 158.05, 154.25,

150.2, 146.3, 144.5, 137.1, 135.4, 135.2, 133.9, 129.7, 128.8, 128.5, 127.8, 127.7, 126.7, 124.5, 124.0, 123.96, 113.15, 88.5, 85.8, 80.8, 74.7, 71.1, 62.65, 54.9.

1-[3'-Benzoyl-2'-nicotinoyl-β-D-furanosyl]-1,2,4-triazole-3-N-carboxamide (50)

1-[5'-0-dimethoxytrityl-3'-benzoyl-2'-nicotinoyl-β-D-furanosyl]-1,2,4-triazole-3-N-carboxamide (5.5 g, 7.3 mmol) was dissolved in 47 mL 80% of glacial acetic acid and the solution was stirred for 2 hrs at room temperature. The TLC did not show starting material. The reaction was neutralized by adding slowly solid NaHCO3 and then water (200 mL). The neutral water solution was extracted with ethyl acetate (3 x 200 mL). The organic layer was washed with water 200 mL and dried with MgSO4. The solvent removal gave crude product (5.2 g), slightly impure.

^{1}H-NMR (300 MHz, DMSO): δ 9.04(1H,d,J=2 Hz), 9.02(1H,s), 8.82(1H,dd,J=4.9, 1.65 Hz), 8.24(1H,dt,J_t=2 Hz,J_d=8 Hz), 8.00(2H,s), 7.96(1H,d,J=1.3 Hz), 7.81(1H,s), 7.71(1H,t,J=7.4 Hz), 7.56-7.49(3H,m), 6.69(1H,d,J=4 Hz), 6.17(1H,m), 5.96(1H,t,J=5 Hz), 5.34(1H,t,J=5.5 Hz), 4.64-4.63(1H,m), 3.86-3.77(2H,m).

^{13}C(75 MHz, DMSO): 164.74(CO), 163.42(CO), 160.23(CO), 157.76, 154.18, 150.03, 145.80, 137.0, 133.86, 129.26, 128.89, 128.77, 128.57, 124.40, 123.89, 88.86, 83.61, 74.89, 71.61, 60.85.

1-[3'-benzoyl-2'-trigonellinyl-β-D-furanosyl]-1,2,4-triazole-3-N-carboxamide (51)

Compound (50) (5.2 g, 0.011 m) was suspended in acetone (50 mL) and methyl iodide (6.5 g, 10.04 m) was added and the mixture was refluxed for 2 hrs. The yellow solution, containing oily residue was evaporated and the residue was tritrated with diethyl ether. Yellow solid (3.75 g) was obtained by filtration.

^{1}H-NMR (300 MHz, DMSO): δ 9.60(1H,s), 9.24(1H,d,J=6 Hz), 9.02(1H,s), 8.98-8.93(1H,m), 8.30-8.25(1H,m), 7.97-7.95(3H,m), 7.71(1H,m), 7.69-7.66(1H,m), 7.56-7.49(1H,d,J=3.8 Hz), 6.17-6.14(1H,m), 5.93(1H,t,J=5 Hz), 5.29(1H,t,J=5.5 Hz), 4.66-4.69(1H,m), 4.43(3H,s), 3.81-3.69(2H,m).

^{13}C-NMR (75 MHz, DMSO): 164.70, 160.40, 160.10, 157.64, 146.65, 145.72, 144.62, 133.85, 129.34, 128.80, 128.73, 128.40, 128.01, 127.87, 88.47, 83.26.

1-[3'-benzoyl-2'-(1,4-dihydrotrigonellinyl-β-D-furosyl]-1,2,4-triazole-3-N-carboxamide (52,AVS 5756)

To the suspension of (7) (4.0 g) in a mixture of 80 mL of water and methylene chloride (80 mL) 2.26 g of sodium bicarbonate and 4.7 g of sodium dithionite was added at 0°C under stream of argon. Upon addition of reducing agents the solid dissolved. After 7 hrs, the two layers were separated, the organic layer was removed and water extracted with one portion of methylene chloride. After drying with $MgSO_4$ the solvent was removed and the residue was treated with 40 mL of diethyl ether. Yellow solid (2.5 g) was collected. The ^{1}H-NMR and TLC showed some impurities therefore it was purified on alumina neutral, eluting with 1% MeOH in $CHCl_3$. Obtained 0.92 g. Trituration with ether gave 0.60 g of yellow crystals.

λ_{max} (MeOH): 206, 364 nm.

^{1}H-NMR (300 MHz, DMSO) of nonpurified compound: 8.93(1H,s), 8.05(2H,d,J=7 Hz), 7.89(2H,m), 7.73-7.57(4H,m), 6.92(1H,s), 6.32(1H,d,J=4 Hz), 5.84(1H,t,J=5 Hz), 5.76-5.72(1H,m), 5.25(1H,m), 4.68-4.47(2H,m), 3.73(2H,m), 2.81(2H,s), 2.74(3H,s),

2',3',5'-Trinicotinoylribavirin (53)

1.22 g (5 mmol) of ribavirin was suspended in 20 mL dry pyridine and 3.3 g (18 mmol) nicotinoyl chloride hydrochloride was added to the suspension along with a catalytic amount of DMAP. The reaction mixture was stirred at 80°C for 24 hours. 1.6 g (9 mmol) nicotinoyl chloride hydrochloride was subsequently added to the reaction mixture and the solution stirred at 80°C overnight. The reaction mixture was then poured into 200 mL of ice water and stirred at room temperature for one hour. The white precipitate was filtered and washed with cold water. It was then crystallized from ethyl alcohol. Yield = 1.96 g, 70%, m.p. 168-170°C; R_f: 0.57 in n-BuOH:AcOH:H_2O = 4:1:1; λ = 264 nm (in EtOH);

Anal: C, H, N; $(C_{26}H_{21}N_7O_8)_2.C_5H_4N$ (1/2 pyridine of crystallization) C, 57.19 (57.66); H, 3.87 (3.62); N, 17.55 (17.59);

^{1}H-NMR (DMSO-d_6): δ 9.23(s,1H,triazole), 9.2-9.06(m,3H,py C-2 protons), 8.9-8.8(m,3H,py C-6 protons), 8.4-8.23(m,3H,py C-4 protons), 7.66-7.43(m,3H,py C-5 protons), 6.88(d,1H,1'H).

Ribavirin 2',3',5'-tritrigonellinate (54)

0.56 g (mmol) of 2',3',5'-trinicotinoylribavirin was dissolved in 20 mL of dry acetone. 0.22 mL (0.51 g, 3.6 mmol) of methyl iodide was added to the solution. The reaction mixture was refluxed overnight. Yellow crystals appeared after cooling of the reaction mixture. The crystals were removed by filtration, washed with cold acetone and dried.

Yield = 0.94 g (95%), m.p. 128-130oC, decomp: 200°C; λ_{max} = 266, 318 nm (in EtOH);

Anal: $C_{29}H_{30}I_3N_7O_8$: 985.32 C 35.45, (35.59); H 3.07, (3.10); N 9.95, (9.80); I 39.64 (39.45).

^{1}H-NMR (DMSO-d_6): δ 9.8-9.66(m,3H,py C-2 protons), 9.36(s,1H,triazole 5H), 9.38-9.26(m,3H,py C-6 protons), 9.1-9.03(m,3H,py C-4 protons), 9.56-9.23(m,3H,C-5 protons), 7.11(d,1H,1'H), 4.56(s,9H,N+CH3).

Ribavirin 2',3',5'-tri(1,4-dihydrotrigonellinate) (55)

0.49 g (0.5 mmol) of (55) was dissolved in 10 mL of degassed water. 0.34 g (4 mmol) of sodium hydrogen carbonate and 0.7 g (4 mmol) of sodium dithionite was added over a period of five minutes to the solution which was stirred at room temperature under argon. 30 mL Dichloromethane was then added to the reaction mixture and after 2.5 hours the reaction mixture was worked up, since the quaternary salt was undetectable in the solution. The organic layer was separated, washed with 5% sodium hydrogen carbonate solution and dried over magnesium sulfate under argon. The solvent was removed under reduced pressure to yield a yellow powder. Yield = 0.18 g (60%), m.p. 98-102oC; λ_{max} = 356 nm (in EtOH);

R_f = 0.77 in $CHCl_3$:acetone = 8:2;

Anal: $C_{29}H_{33}N_7O_8.2H_2O$ C, 54.11 (54.01); H, 5.79 (5.30); N, 15.23 (14.84).

^{1}H-NMR (DMSO-d_6): δ 9.16(s,1H,triazole 5-proton), 7.16(d,2H,carbamoyl), 3-2.66(m,15H,C-4 protons, N-CH_3).

1-(5'-dimethoxytrityl-2',3'-bis-0-nicotinylate-β-D-ribofuranosyl)-1,2,4-triazole-3-N-carboxamide (56)

Ribavirin-DMT (0.55 g, 0.001 mol) (7) was dissolved in 10 mL of methylene chloride and triethylamine (0.121 g, 0.0012 mol), catalytic amount of N,N-dimethyl-4-aminopyridine, and nicotinic anhydride (0.38 g, 0.002 mol) were added. The reaction was stirred for 30 minutes and controlled by using TLC if all starting material disappeared. Then 50 mL of 5% $NaHCO_3$ was added and the mixture extracted with methylene chloride (3 x 15 mL); organic layer was separated, washed with water (30 mL) and dried ($MgSO_4$). Pure product was obtained as a yellow solid (yield 91%). Elemental analysis obtained for this product correspond to the dihydrate. Calculated for $C_{41}H_{36}N_6O_9$ x 2 H_2O:C, 62.11; H, 5.08; N, 10.60. Found: C, 61.72; H, 4.60; N, 10.54. ^{1}H-NMR ($CDCl_3$)(300 MHz, FT MODE) δ(PPM): 9.17(1h,s), 9.10(1H,s), 8.77(1H,d,J-4.9 Hz), 8.75(1H,d,J=4.9 Hz), 8.59(1H,d,J=4.4 Hz), 8.20-8.16(2H,m), 7.46(2H,d,J=7.3 Hz), 7.38-7.19(10H,m), 6.82-6.75(5H,m), 6.44(2H,d,J=2.7 Hz), 6.06-6.03(1H,m), 4.64-4.62(1H,m), 3.74(6H,s), 3.74-3.53(2H,m). ^{13}C-NMR ($CDCl_3$)(75 MHz FT mode) δ(ppm): 163.7, 163.5, 160.3, 158.5, 157.4, 154.05, 145.0, 144.0, 127.1, 137.0, 135.1, 130.1, 130.0, 129.95, 128.0, 127.85, 126.9, 124.6, 124.3, 123.4, 123.38, 113.1, 113.06, 89.6, 86.9, 82.7, 74.9, 72.3, 62.7, 55.05.

1-(2,3,5-tri-0-propionyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (57)

Ribavirin (4.88 g, 0.02 mol) was dissolved in 50 mL of anhydrous pyridine. Propionic anhydride (9.2 mL, 9.66 g, 0.074 mol) was added to the solution and the reaction mixture stirred at room temperature overnight. The pyridine was subsequently removed in vacuo. Trace were removed by azeotropic distillation with toluene. The oily residue was partitioned between ethyl acetate and 5% sodium bicarbonate solution and washed with 5% sodium bicarbonate twice and with water once. The aqueous solution was backwashed with ethyl acetate. The combined organic phases were then evaporated in vacuo yielding a yellow oil (7.2 g, 87%).

^{1}H-NMR ($CDCl_3$): δ 8.53(1H,s,triazole), 6.13(1H,d,1'-H), 7.36(2H,d,NH_2), 5.56-5.9(2H,m,2'-H and 3H), 4.42(3H,m,5'-H,4'-H), 1.16(9H,t,CH_3), 2.33(6H,9,CH_2), TLC silica, R_f = 0.72 n butanol:acetic acid:H_2O:4:1:1.

1-(5'-0-dimethoxytrityl-2',3'-bis-0-isobutyrate-β-D-ribofurnosyl)-1,2,3-triazole-3-N-isobutryl-carboxamide (58)

5'-Dimethoxytrityl ribavirin (7)(24.5 g, 0.045 mol) (7) was dissolved in 100 mL dry pyridine. N,N-dimethylaminopyridine (5.0 g) and isobutyric anhydride (74.3 mL, 0.448 mol) were added and the mixture was stirred at RT for 48 h. It was poured onto 300 mL ice and extracted with chloroform (2x400 mL). The combined organic extracts were washed with 5% $NaHCO_3$ (2x400 mL), water (400 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oil was purified on a silica column with $CHCl_3$:MeOH (40:1) as eluent. This gave 20.0 g (59%) of product as a white solid.
IR cm^{-1} (nujol mull): υ_{NH}3360, $\upsilon_{C=O}$1740, 1710, $\upsilon_{C=N,C=C}$1610.
H-NMR ($CDCl_3$): δ 9.22(s,1H,NH), 8.4(s,1H,5-H), 7.45-6.7(m,13H,arom), 6.1(d,1H,1'H), 5.9(t,1H,3'H), 5.8(t,1H,4'H), 4.45(m,1H,2'H), 3.85(s,6H,2xOCH_3), 3.5-3.1(m,3H,5'CH_2 + isobut CH), 2.65(m,2H, 2x isobut CH), 1.25(m,18H, 6x CH_3).

1-(2',3'-bis-0-isobutyrate-β-D-ribofuranosyl)-1,2,4-triazole-3-N-isobutryl carboxamide (59)

Compound (58) (12.0 g, 0.01586 mol) was dissolved in 60 mL of 80% acetic acid and the mixture was stirred at room temperature for 1 h. It was neutralized with solid sodium bicarbonate (400 g) until no more gas evolved. It was then diluted with 1500 mL water and extracted with chloroform (2 x 500 mL). The organic layer was washed with water (700 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oily solid was triturated with petroleum-ether to give 5.5 g (76.3%) of the product as a white solid.
IR cm^{-1} (nujol mull): υ_{OH}3520, υ_{NH}3110, $\upsilon_{C=O}$1750, 1730, 1700, $\upsilon_{C=N}$1660,1540.
H-NMR ($CDCl_3$): δ 9.48(s,1H,NH), 8.7(s,1H,5-H), 6.1(d,1H,1'H), 5.75(t,1H,3'H), 5.6(t,1H,4'H), 4.35(m,1H,2'H), 4.05-3.8(m,2H,5'CH_2), 3.5(m,2H,OH + isobut CH), 2.6(m,2H, 2 x isobut CH), 1.25(m,18H, 6 x CH_3).

1-[5'(3-carbonylpyridine)-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl]1,2,4-triazole-3-N-isobutryl carboxamide (60)

Compound (59) (7.5 g, 0.0165 mol) was dissolved in 90 mL dry pyridine and the

solution was cooled to 0°C. Nicotinic anhydride (8.9 g, 0.0392 mol) was added to it and the mixture was stirred overnight at room temperature. It was poured onto 200 mL ice and extracted with chloroform (2 x 300 mL). The combined organic extracts were washed with 5% $NaHCO_3$ (2 x 300 mL), water (300 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the resulting oil was dissolved in a small amount of ether and evaporated. This gave 6.0 g (65%) of product as a white solid.
IR cm^{-1} (nujol mull): υ_{NH}3380, $\upsilon_{C=O}$br 1760-1700, $\upsilon_{C=N,C=C}$1600.
^{1}H-NMR ($CDCl_3$): δ 9.48(s,1H,NH), 9.2(s,1H,Pyr), 8.8(d,1H,Pyr), 8.54(s,1H,5-H), 8.4(d,1H,Pyr), 7.5(m,1H,Pyr), 6.15(d,1H,1'H), 5.82(m,1H,3'H), 5.75(t,1H,4'H), 4.75(d,1H,2'H), 4.6(m,2H,5'CH_2), 3.5(m,1H, isobut CH), 2.65(m,2H, 2 x isobut CH), 1.25(m,18H, 6 x CH_3).

1-[5'-(1-methyl-3-carbonyl pyridinium)-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl]-1,2,4-triazole-3-N-isobutryl carboxamide iodide (61)

Compound (60) (4 g, 0.00715 mol) was dissolved in 50 mL dry THF. 4.8 g of methyl iodide was added and the solution was heated at 70°C for 4 h. The THF was decanted off and the remaining oil was washed with 100 mL dry THF. The residual solvent was removed under vacuum. This gave 4.8 g (95.6%) of yellow crystalline product.
UV λ_{max} (MeOH): 266.0, 216.5
IR cm^{-1} (nujol mull): υ_{NH}br 3600-3000, $\upsilon_{C=O}$1740,1720,1700, $\upsilon_{C=N,C=C}$1640,1600.
^{1}H-NMR ($CDCl_3$): δ 9.65(s,2H,NH+Pyr), 9.55(s,1H,Pyr), 9.12(d,1H,Pyr), 8.78(s,1H,5-H), 8.38(m,1H,Pyr), 6.32(d,1H,1'H), 5.9(t,1H,3'H), 5.8(t,1H,4'H), 4.95(d,1H,2'H), 4.76(s,3H,N-CH_3), 4.7-4.5(m,2H,5'CH_2), 3.15(m,1H, isobut CH), 2.65(m,2H, 2 x isobut CH), 1.25(m,18H, 6 x CH_3).

1-[5'-N-methyl-3-carbonyl-1,4-dihydropyridine)-2',3'-bis-0-isobutyrate-β-D-ribofuranosyl]-1,2,4-triazole-3-N-isobutryl carboxamide (62,AVS 5222)

3.6 g (0.005 mol) of quaternary compound (61) was dissolved in 600 mL ice-cold deionized water and washed with 300 mL ethylacetate. The aqueous layer was degassed and cooled to 0°C. A mixutre of sodium bicarbonate (7.6 g, 0.09 mol) and sodium

dithionite (14.8 g, 0.085 mol) was added portionwise to the stirred solution. After 1 h 30 min, it was extracted with ice-cold degassed ethyl acetate (600 mL). The organic layer was washed with ice-cold water (500 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure to give 2.26 g (78.7%) of product.

UV λ_{max} (MeOH): 359.5, 212.0

IR cm^{-1} (nujol mull): υ_{NH}3320-3180, $\upsilon_{C=O}$1750,1730,1700, $\upsilon_{C=N,C=C}$1670,1640,1610.

^{1}H-NMR ($CDCl_3$): δ 9.45(s,1H,NH), 8.5(s,1H,5-H), 7.0(s,1H,Pyr), 6.1(d,1H,1'H), 5.8(t,1H,4'H), 5.65(d,1H,Pyr), 5.55(t,1H,3'H), 4.75(m,1H,Pyr), 4.55(m,2H,5'CH_2), 4.28(m,1H,2'H), 3.5(m,1H, isobut CH), 3.05(brS,2H,PyrC_4), 2.94(s,3H,N-CH_3), 2.6(m,2H, 2 x isobut CH), 1.25(m,18H, 3 x CH_3).

1-(2',3'-bis-0-acetate-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (63)

Compound (62) (8.0 g, 0.01319 mol) was dissolved in 50 mL of 80% acetic acid and the mixture was stirred at room temperature for 1 h. It was neutralized with solid sodium bicarbonate until no more gas evolved, and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure to give 1.5 g (37.5%) of the product as a white solid.

IR cm^{-1} (nujol mull): υ_{OH+NH}3500,3350,3180, $\upsilon_{C=O}$1750,1690, $\upsilon_{C=N}$1670,1620.

^{1}H-NMR ($CDCl_3$): δ 8.9(s,1H,5-H), 7.95(s,1H,NH), 7.72(s,1H,NH), 6.25(d,1H,1'H), 5.7(t,1H,3'H), 5.48(t,1H,4'H), 5.18(t,1H,OH), 4.25(m,1H,2'H), 3.75-3.5(m,2H,5'CH_2), 2.05(2s,6H,2xCH_3).

1-[5'-(3-carbonylpyridine)-2',3'-bis-0-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-N-nicotinoyl carboxamide (64)

Compound (63) (2.2 g, 0.00755 mol) was dissolved in 100 mL dry methylene chloride, to it triethylamine (0.84 g, 0.008305 mol) and nicotinic anhydride (1.9 g, 0.008305 mol) were added. The mixture was stirred at room temperature for 36 h, washed with 5% $NaHCO_3$ (100 mL) and H_2O (100 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. This gave 1.2 g (30.9%) of product as a pale brown solid.

IR cm^{-1} (nujol mull): υ_{NH}3380, $\upsilon_{C=O}$br 1750-1700, $\upsilon_{C=C,C=N}$1650,1600.

^{1}H-NMR ($CDCl_3$): δ 10.3(s,1H,NH), 9.15(s,2H,2xPyr), 8.82(d,1H,Pyr), 8.75(d,1H,Pyr), 8.5(s,1H,5-H), 8.35(d,1H,Pyr), 8.22(d,1H,Pyr), 7.5(m,2H,Pyr), 6.18(d,1H,1'H), 5.85(m,1H,3'H), 5.75(t,1H,4'H), 4.8-4.55(m,3H,2'H+5'CH_2), 2.15(2s,6H,2xCH_3).

1-[5'-(1-methyl-3-carbonyl pyridinium)-2',3'-bis-0-acetate-β-D-ribofuranosyl]-1,2,4-triazole-3-N-(N-methylnicotinoyl)carboxamide diiodide (65)

Compound (64) (1.0 g, 0.001948 mol) was dissolved in 50 mL dry THF and methyl iodide (4 mL) was added to it. The mixture was refluxed overnight, cooled and the solid was filtered and washed with cold THF to give 1.2 g (77.5%) of yellow crystalline product.

UV λ_{max} (MeOH): 265.0, 219.5

IR cm^{-1} (nujol mull): υ_{NH}br 3600-3000, $\upsilon_{C=O}$1750,1720,1700, $\upsilon_{C=C,C=N}$1640,1590.

^{1}H-NMR (DMSO-d_6): δ 11.95(br,s,NH), 9.6(s,Pyr), 9.55(s,Pyr), 9.33(d,Pyr), 9.25(d,Pyr), 9.15(s,5-H), 9.05(m,Pyr), 8.95(d,Pyr), 8.3(m,Pyr), 6.55(d,1'H), 5.8(m,3'H+4'H), 4.85-4.5(m,2'H+5'CH_2), 4.48(2s,2xN-CH_3), 2.1(2s,2xCH_3).

Ribavirin percarbanilate (66)

Ribavirin (112 mg, 0.5 mmol) was dissolved in 3.5 mL of dry pyridine contained in a 13 mm x 100 mm culture tube with Teflon-lined screw cap. Phenyl isocyanate (200 μL) was added. The tube was capped, and the mixture was heated at 80°C for 1 h in a dry block reactor. The solvent was removed under water aspirator vacuum. The sticky residue was dissolved in 20 mL $CHCl_3$ (cloudy) and washed with water (3x). The $CHCl_3$ was dried with anhydrous Na_2SO_4, filtered, and evaporated under vacuum to give a sticky yellow oil. The oil was dissolved in 2 mL ethyl acetate and 1 mL ethanol. Hexane (1 mL) was added dropwise with stirring and a white solid precipitated from solution. The crystals were filtered and dried under vacuum (yield: 327 mg). The melting range was 184°C-187°C (ribavirin: 167°C-168°C). The material gave 4 spots on silica gel TLC in $CHCl_3$:isopropanol (95:5). The main spot had an R_f of 0.79; other components had R_f values of 0.3, 0.6, and 0.94. The UV spectrum contained bands at 206 nm and 250 nm (ε_{250} = 4 x 10^5). In a 50% methanol mobile phase, the derivative gave 2 sharp peaks

that eluted at 4.5 min (peak height = 6411) and 11.3 min (peak height = 2862) on the Perkin Elmer Pecosphere ODS column system (250 nm).

IN VITRO STUDY

Blood: Trunk blood was obtained from a freshly killed Sprague-Dawley rat and collected in a 15 mL polypropylene tube which contained heparin (1000 units/mL; 200 μL/tube). The tube was then vortexed for 30 s and snap frozen at -80°C to hemolyze red cells.

Tissue homogenates: The tissue of interest was obtained from freshly killed rats and was homogenized in isotonic phosphate buffer to generate a 20% (w/v) suspension. The homogenate was centrifuged for 5 min and the supernatant used immediately.

Procedure: 5.0 mL of blood or tissue homogenate was added to a 20 mL vial and incubated for 5 min in a shaking water bath (37°C). to μL of a dihydropyridine stock solution (5 mM) made up in methanol was then added to the homogenate or blood as the system was shaken for 5 sec. At selected time post-addition, the vial was vortexed and 300 μL of sample removed and mixed with 600 μL of ice-cold acetonitrile (04 92:8 acetonitrile:DMSO in the case of blood). The samples were vortexed for 20 sec and centrifuged in a Beckman Microfuge 12. The supernatant is then removed as assayed by HPLC.

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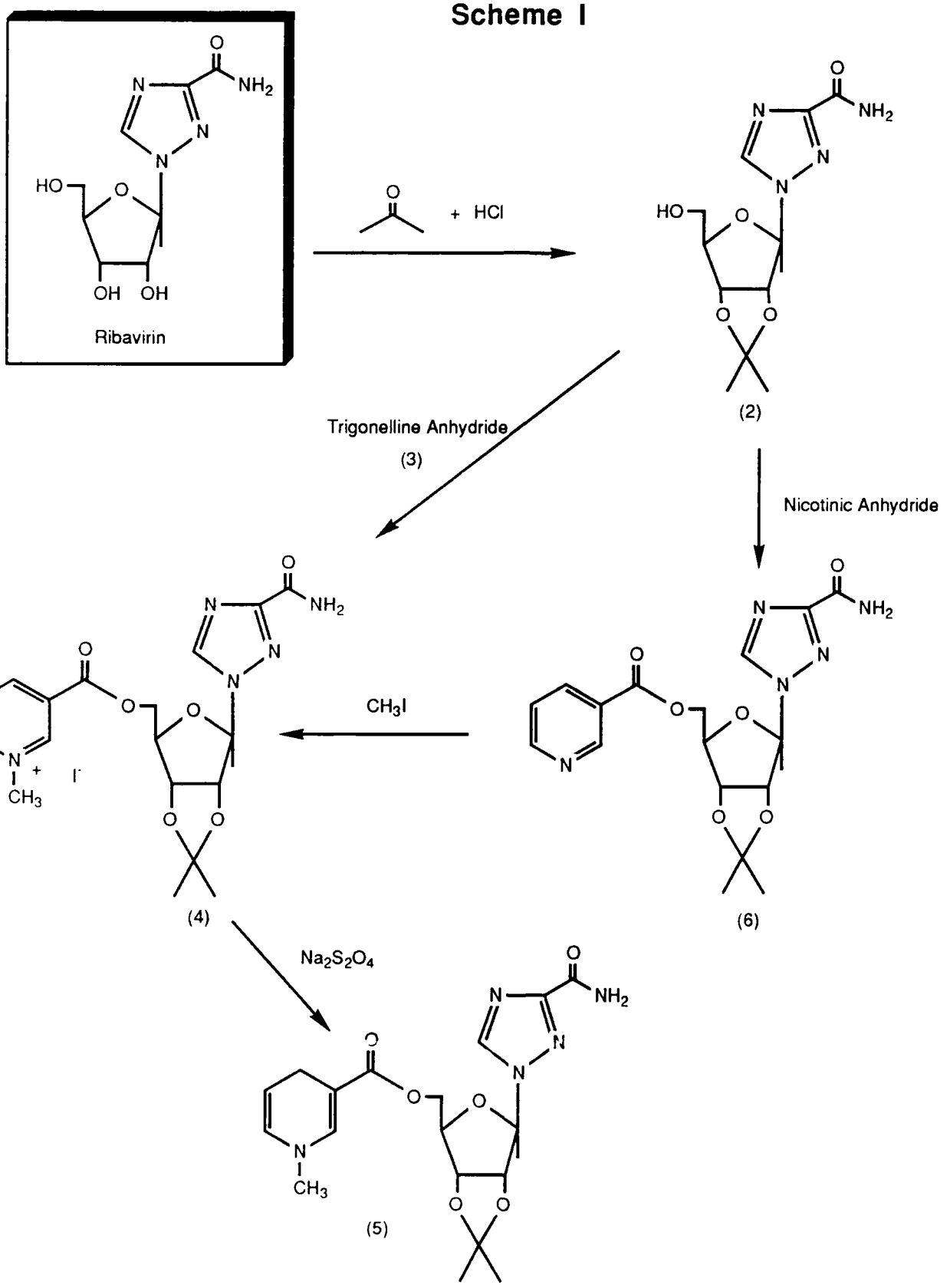
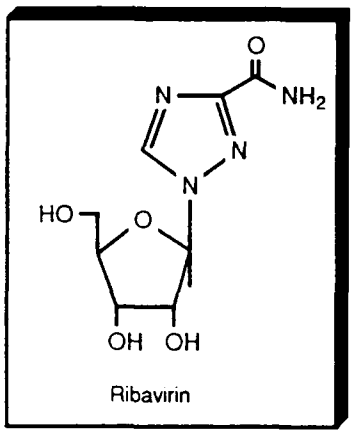
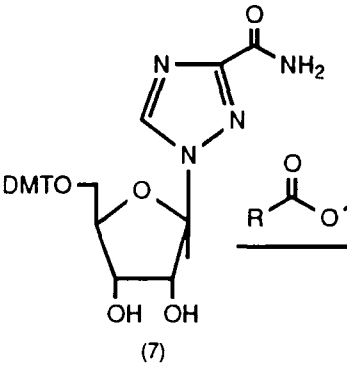
Scheme I

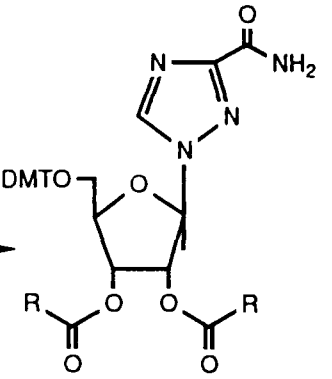

Ribavirin
+ HCl
(2)
Trigonelline Anhydride
(3)
Nicotinic Anhydride
CH_3I
(4)
(6)
$Na_2S_2O_4$
(5)

Scheme II

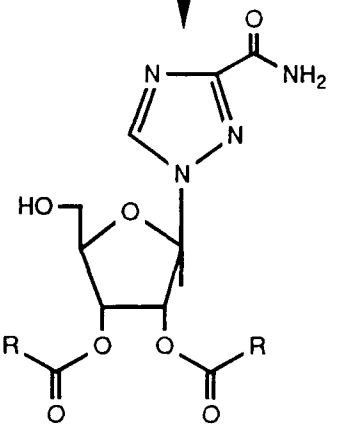


DMT-Cl

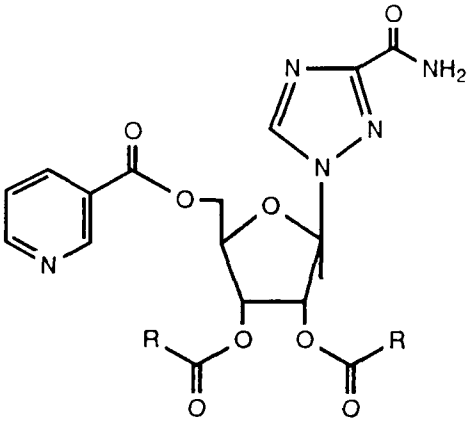

(7)


(8) R = pivaloate; (9) R = benzoate
(10) R = isobutyrate; (11) R = acetate

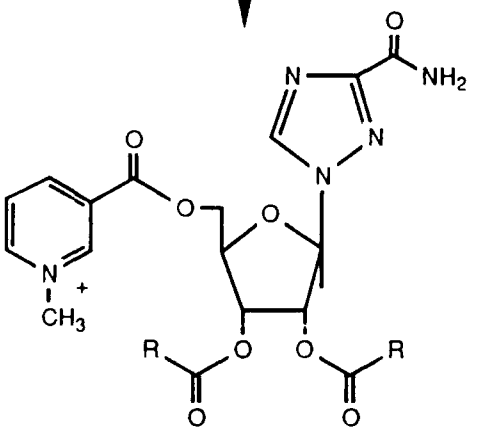
CH_3COOH

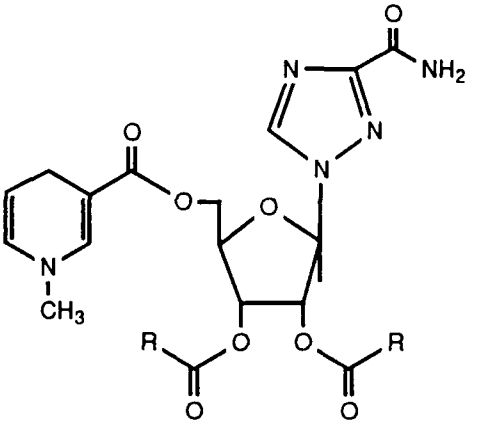

(12) R = pivaloate; (13) R = benzoate
(14) R = isobutyrate; (15) R = acetate

Nicotinic Anhydride

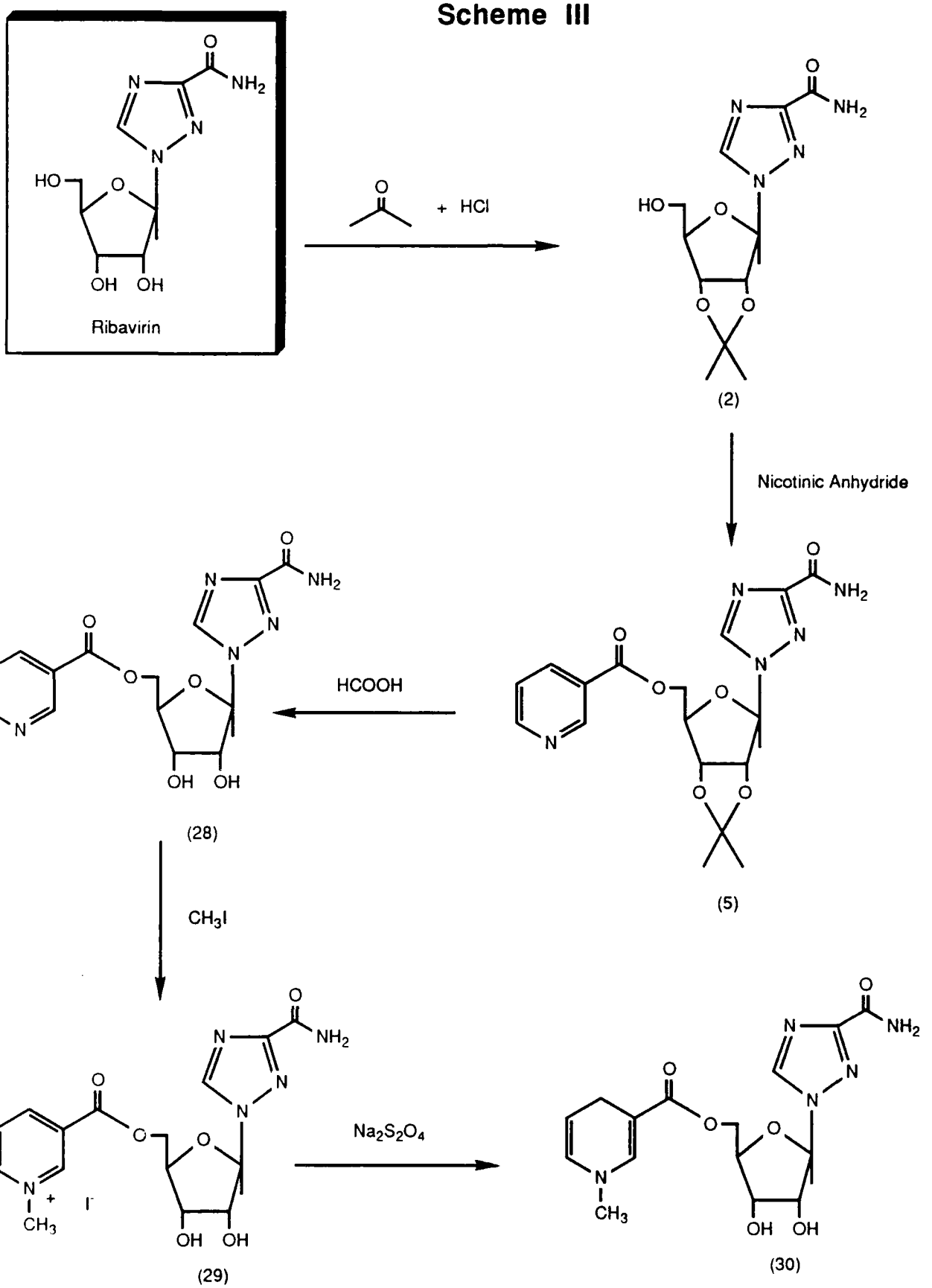

(16) R = pivaloate; (17) R = benzoate
(18) R = isobutyrate; (19) R = acetate

CH_3I

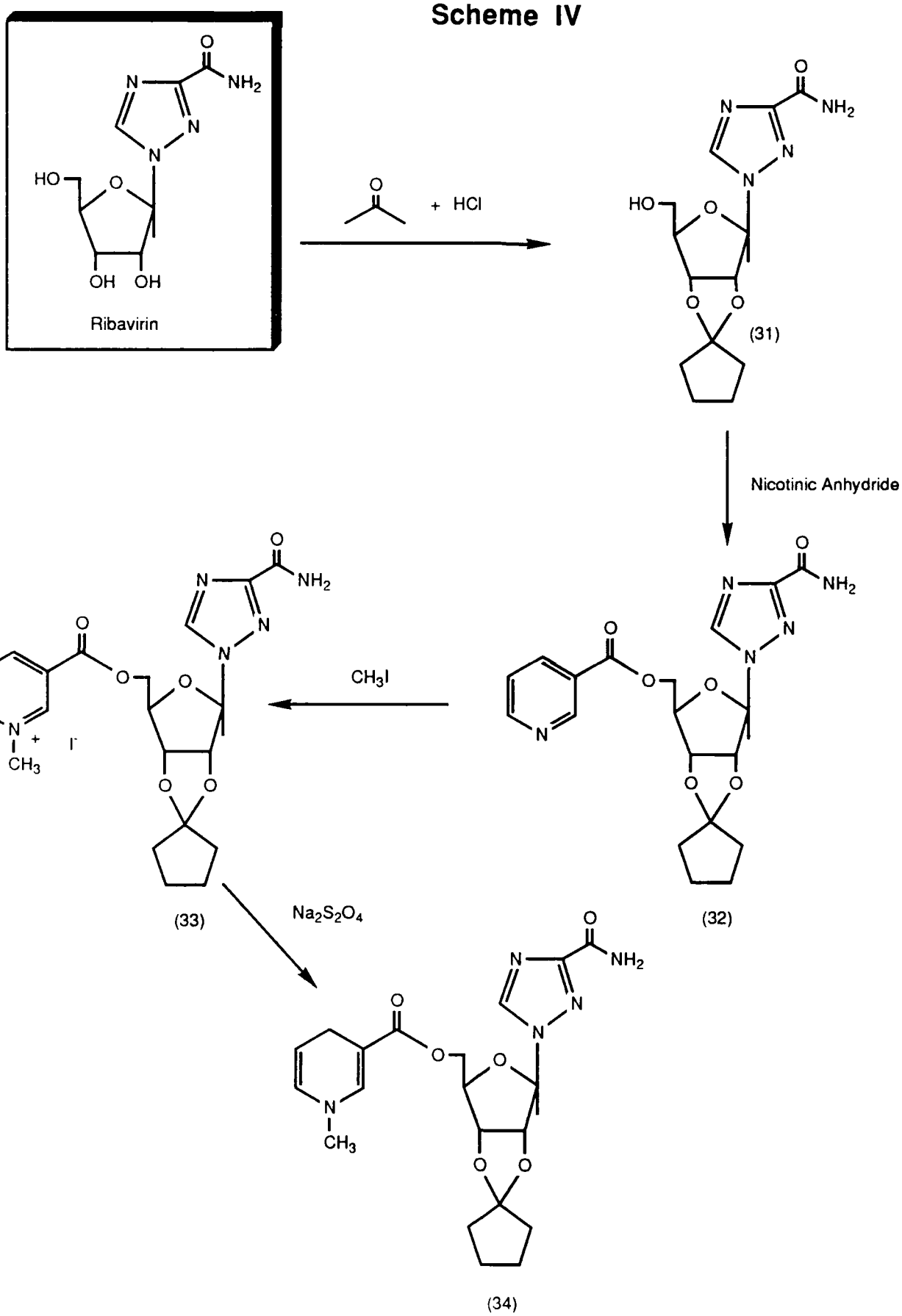

(20) R = pivaloate; (21) R = benzoate
(22) R = isobutyrate; (23) R = acetate


(24) R = pivaloate; (25) R = benzoate
(26) R = isobutyrate; (27) R = acetate

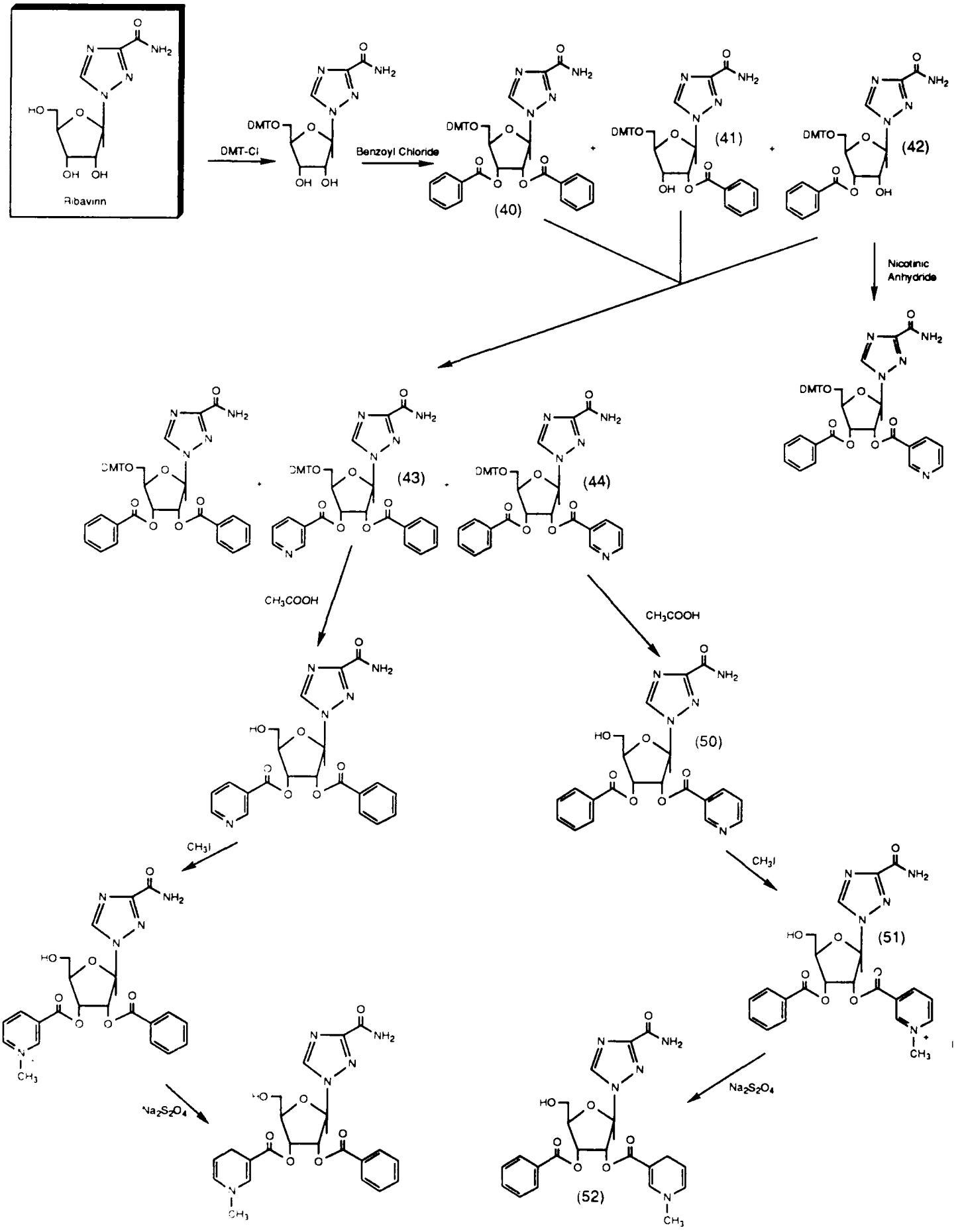
Scheme III


Ribavirin
+ HCl
(2)
Nicotinic Anhydride
(5)
HCOOH
(28)
CH_3I
(29)
$Na_2S_2O_4$
(30)

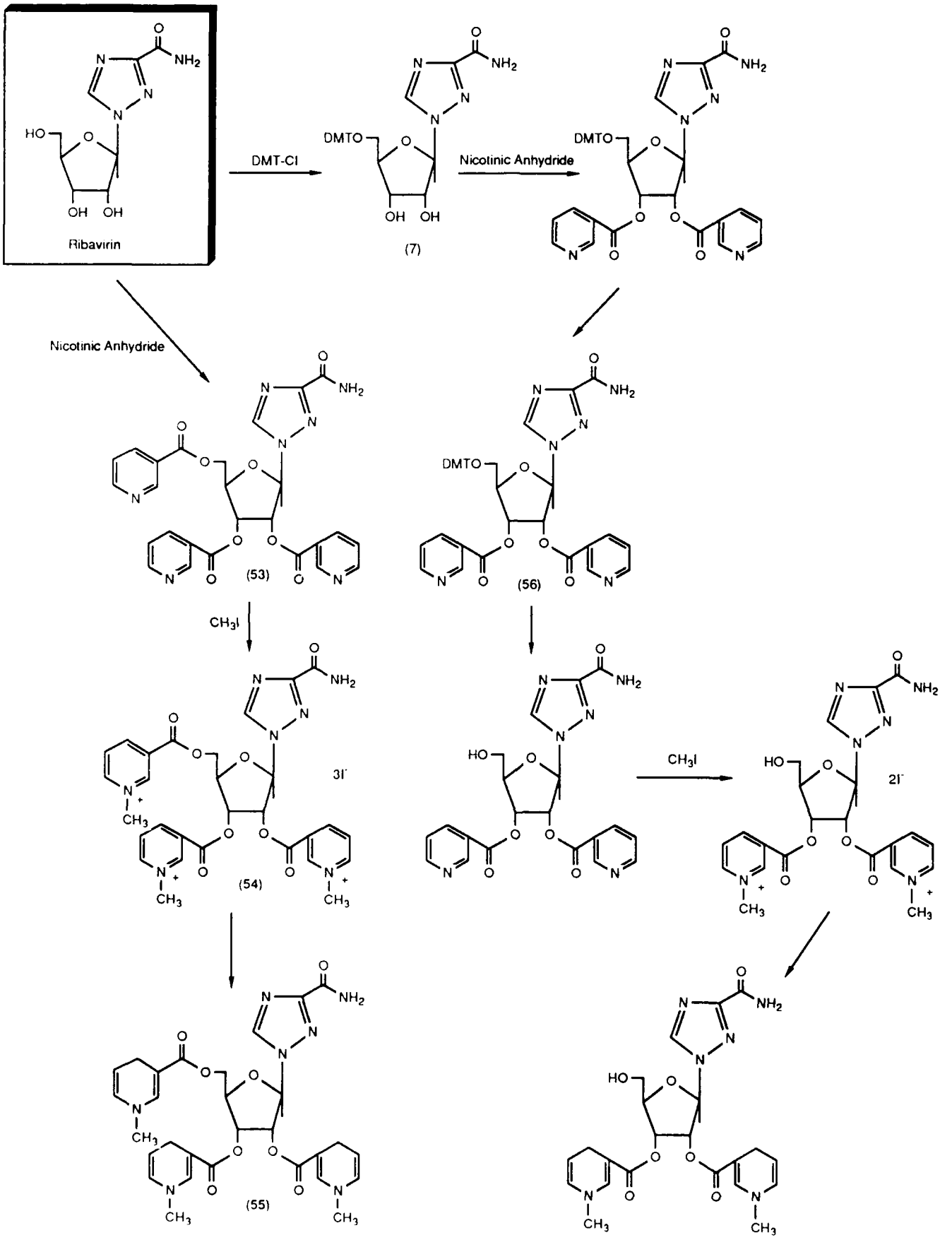
Scheme IV



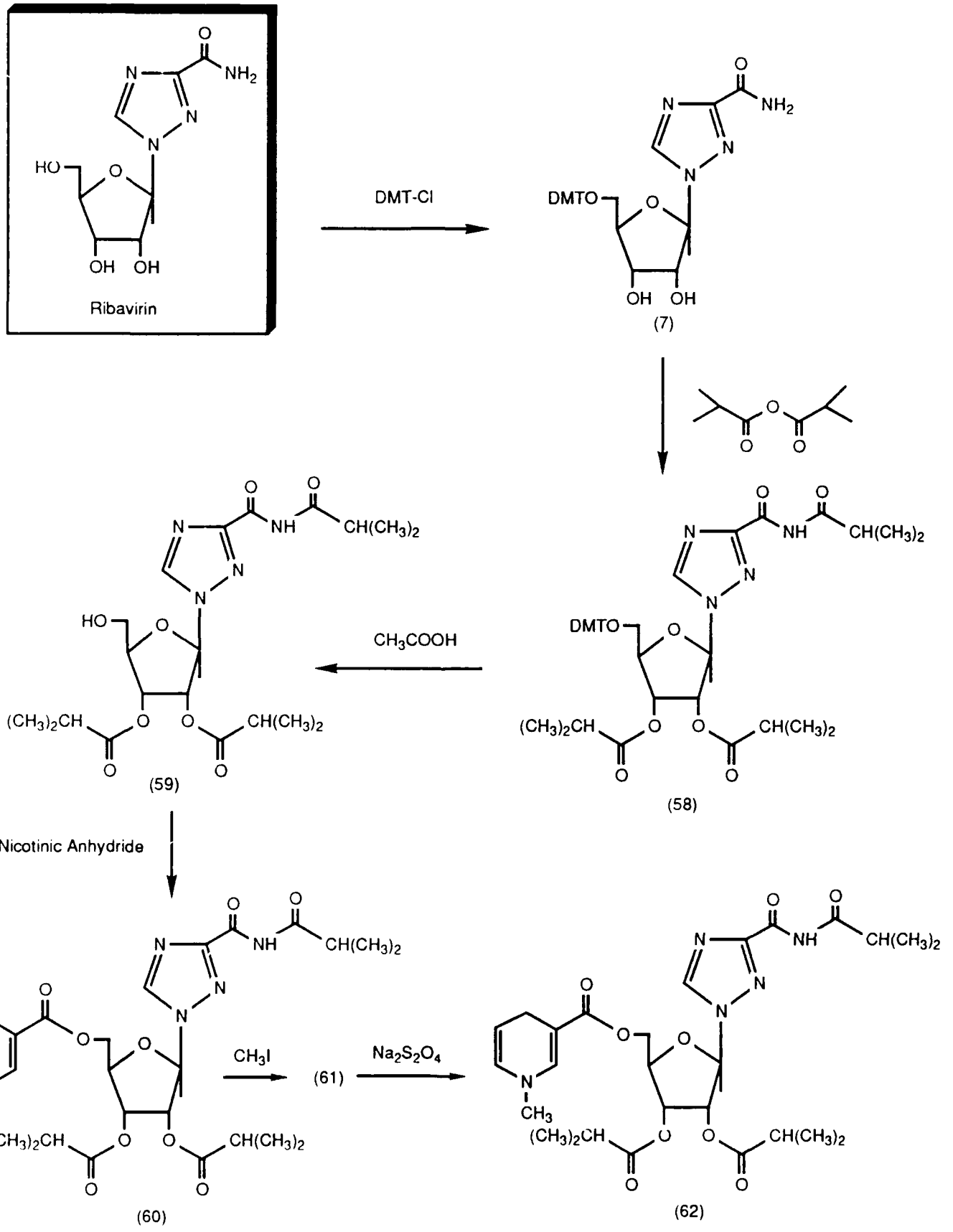
Scheme V


Ribavirin
DMT-Cl
Benzoyl Chloride
(40)
(41)
(42)
Nicotinic Anhydride
(43)
(44)
CH3COOH
CH3COOH
(50)
CH3I
CH3I
(51)
Na2S2O4
Na2S2O4
(52)

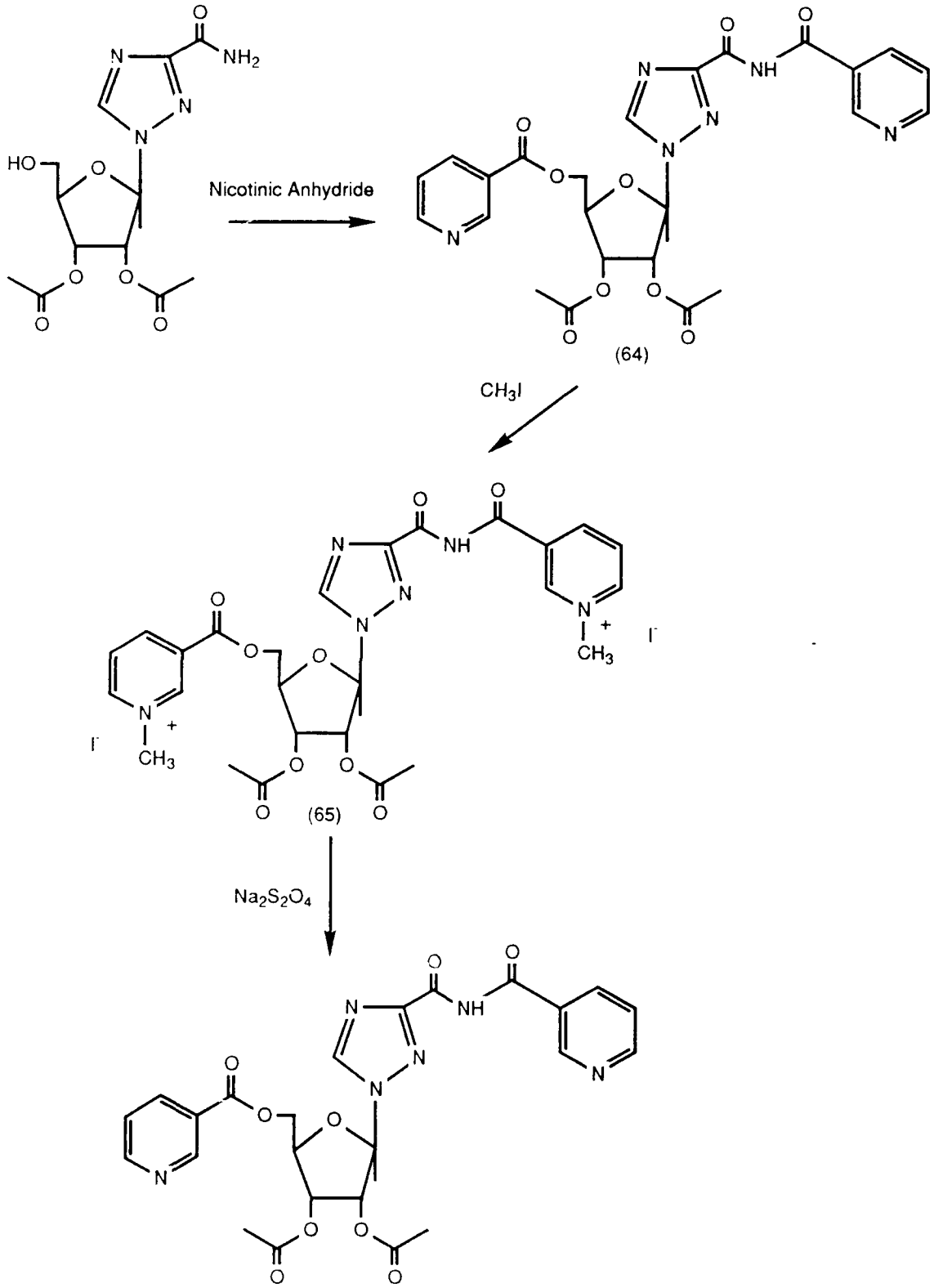
Scheme VI


Ribavirin
DMT-Cl
(7)
Nicotinic Anhydride
Nicotinic Anhydride
(53)
(56)
CH_3I
3I⁻
(54)
CH_3I
2I⁻
(55)

Scheme VII


Ribavirin
DMT-Cl
(7)
(58)
(59)
CH3COOH
Nicotinic Anhydride
(60)
CH3I
(61)
Na2S2O4
(62)

Scheme VIII



Scheme IX

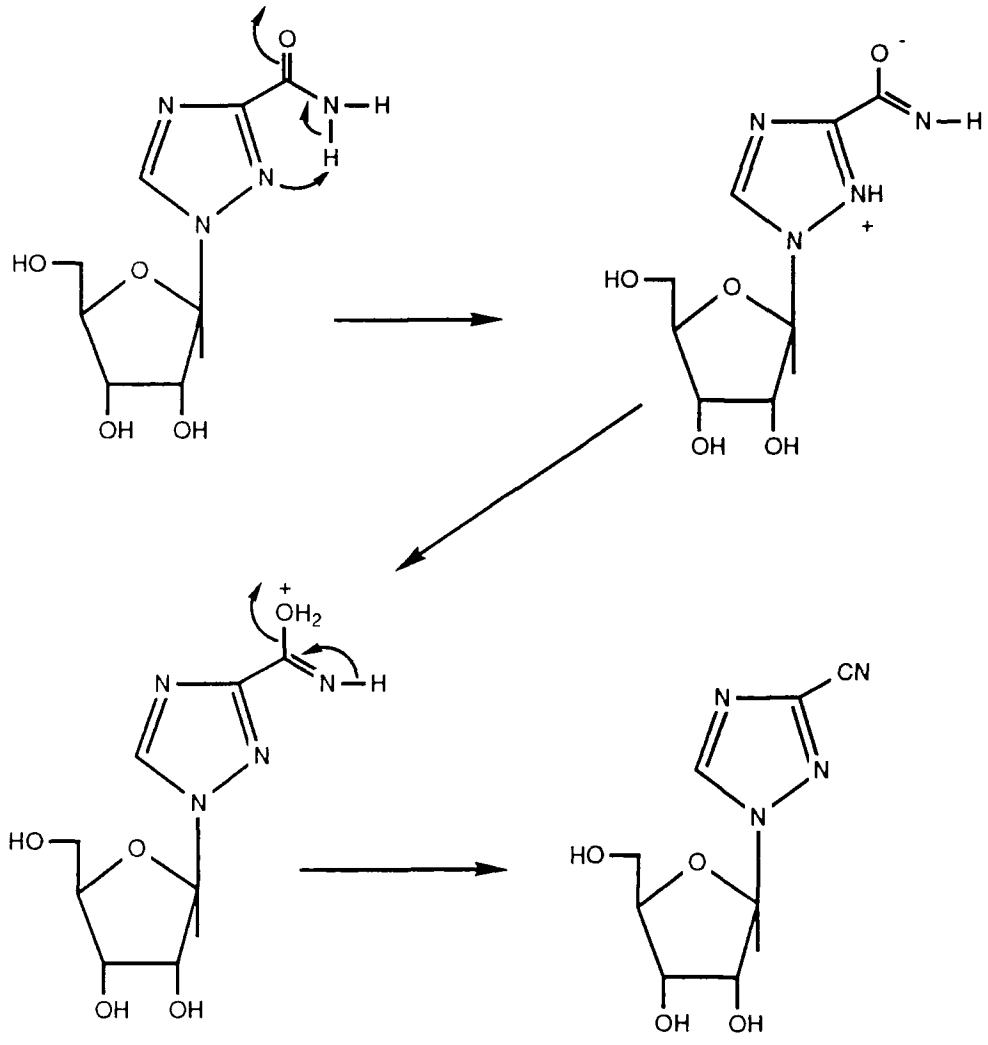


Figure 1

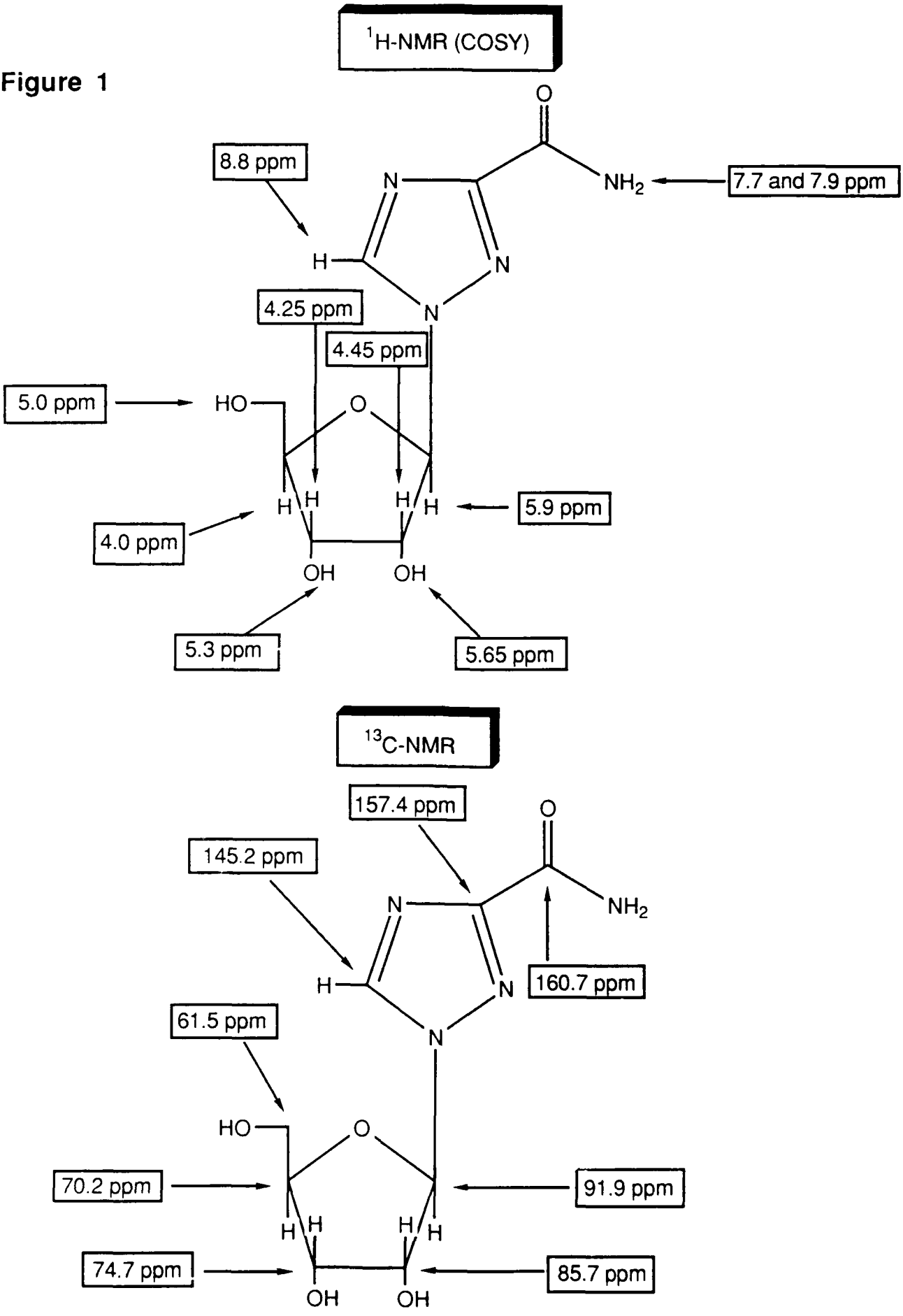

^{1}H-NMR (COSY)
8.8 ppm
7.7 and 7.9 ppm
4.25 ppm
4.45 ppm
5.0 ppm
4.0 ppm
5.9 ppm
5.3 ppm
5.65 ppm
^{13}C-NMR
157.4 ppm
145.2 ppm
160.7 ppm
61.5 ppm
70.2 ppm
91.9 ppm
74.7 ppm
85.7 ppm

Figure 2. ^1H - ^1H NMR spectra (cosy) of ribavirin

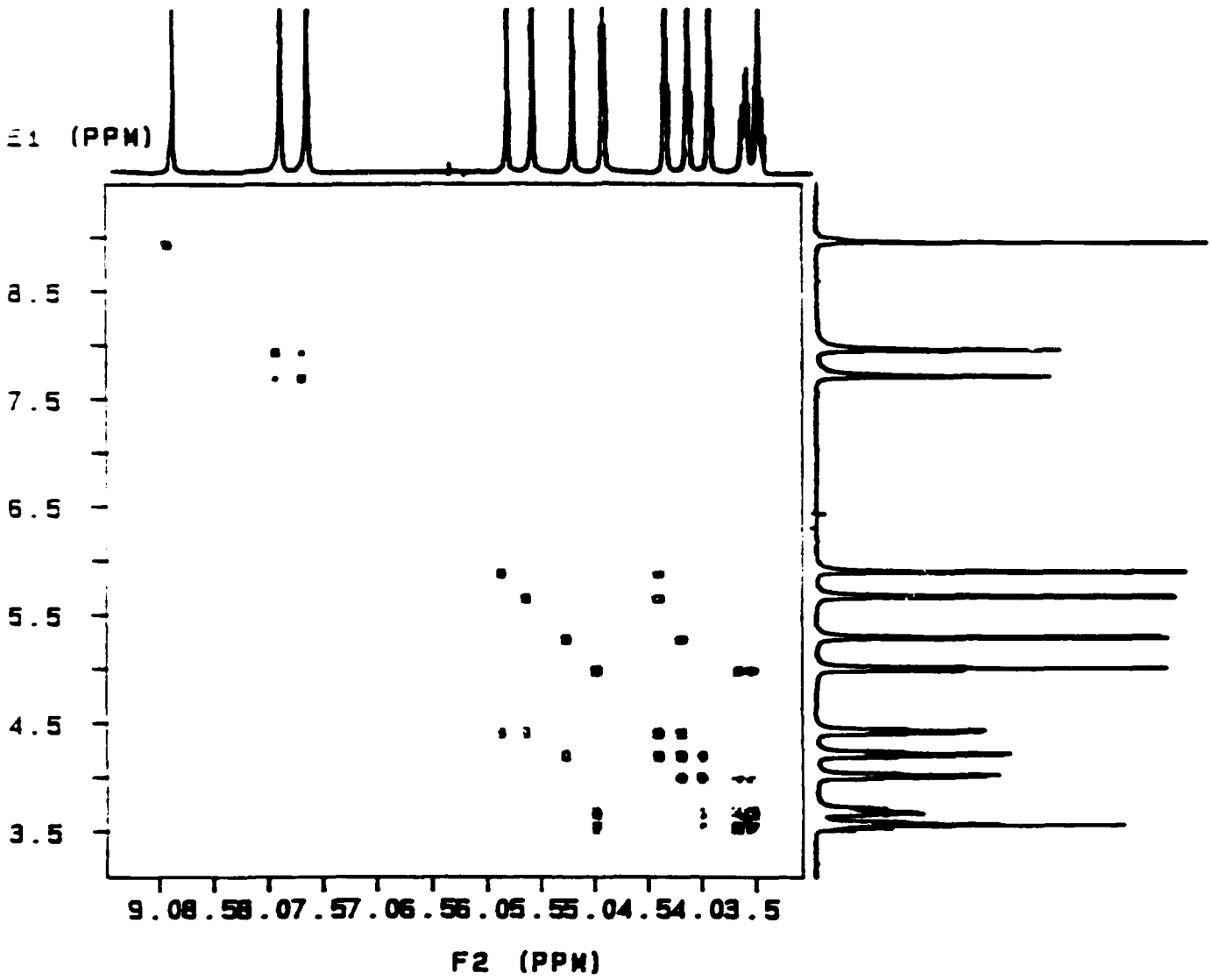


Figure 3. Isomerization of monoacylated DMT-protected ribavirin

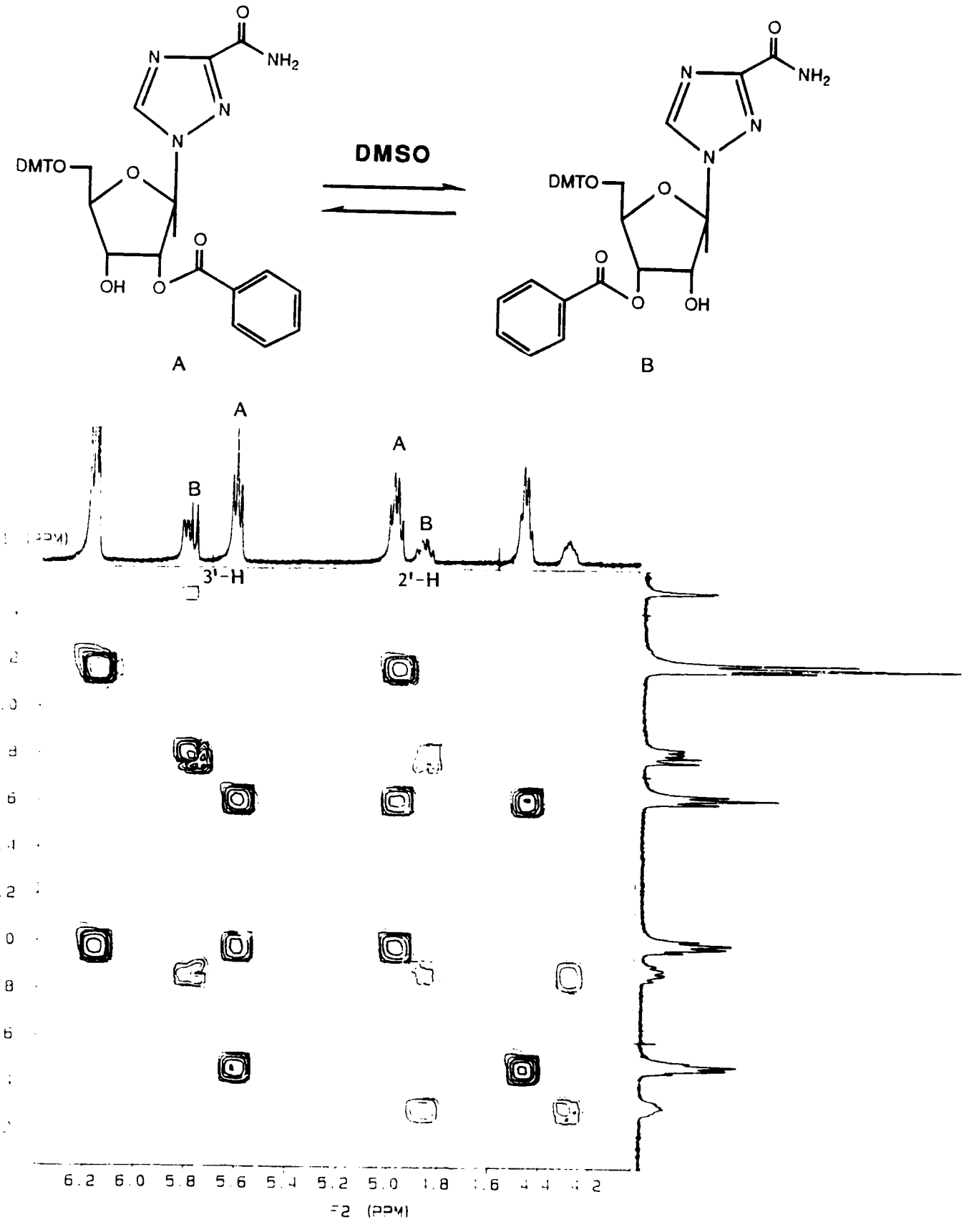


TABLE I. In Vitro Stability of Ribavirin CDS's in Various Biological Matrices

Matrix	Compound	Half-life (min)	Correlation Coefficient (r)
Brain Homogenate (20%, rat)	(5)	16.4	0.991
	(25)	8.7	0.989
	(24)	11.0	0.999
	(26)	23	0.990
Liver Homogenate (20%, rat)	(5)	10.2	0.985
	(25)	< 1	-
	(24)	< 1	-
	(26)	< 1	-
Whole Blood (Rat)	(5)	14.6	0.990
	(25)	< 1	-
	(24)	20.0	0.997
	(26)	3.7	0.980
pH 7.4 phosphate buffer	(26)	197	0.999

TABLE II. Apparent First-Order Rate Constants (sec^{-1}) and Half-Lives for Degradation of (4) at 25°C

Medium	pH	k (sec^{-1})	$t_{1/2}$ (hrs)	r^2
0.1 M HCl	1.099	2.41×10^{-5}	8.0	0.999
Phosphate buffer	6.47	2.23×10^{-5}	8.6	0.960
Phosphate buffer	7.57	1.74×10^{-5}	1.1	0.979

$k_{H+} = 3.032 \times 10^{-4}\ M^{-1}s^{-1}$
$k_{OH-} = 4.121 \times 10^{-2}\ M^{-1}s^{-1}$

TABLE III. Partition Coefficients for Various Ribavirin Derivatives between Acetonitrile and Aqueous Solution at Various pH's

Compound	pH	Partition Coefficient
(4)	7.4	0.372
	3.6	0.360
(2)	7.4	1.07
	3.6	1.04
(28)	7.4	0.0
	3.6	0.0

TABLE IV. In Vitro Stability of (4) in Various Matrices

Matrix	k (sec^{-1})	$t_{1/2}$ (min)	r^2
Phosphate buffer (pH = 7.4)	3.61×10^{-4}	32	0.995
20% Rat Brain Homogenate	3.73×10^{-4}	31	0.979
Whole Rat Blood	7.28×10^{-4}	16	0.996

TABLE V. Acute Lethality of Ribavirin CDS's

Compound	Dose (mg/kg)	% Survival
(5)	68	33
	45.3	100
(25)	75.2	0
	50.1	0
	34	80
	16.7	100
(24)	85.9	67
	57.3	100
	38.2	100
(26)	102.2	100
(62)	100	50
	50	33
	25	100

TABLE VI. Affect of Various Doses of Ribavirin CDS's on the Body Weight of Rats

Drug	Dose (mg/kg)	0	1	2	3	4	5	6	7	8	9
(5)	45.5	342	345	341	344			339	333	340	
	45.5	356	356	362	363			361	360	356	
	45.5	348	349	350	347			347	351	346	
(25)	34	248	241			246	245	243	246		
	34	240	247			255	249	256	263		
	16.7	224	223	231			250	256	266		
(24)	85.8	226	217			218	215	216	221		
	57.2	236	230	237			260	267			
	57.2	236	240	244			270	276	279		
	57.2	237	230	240			261	271	272		
	38	228	234	243			268	275	285		
(26)	102	229	227	236		227	227	233	226		
	102	249	248	255		252	255	257	253		
	102	239	230	239		235	237	238	233		
(62)	25	388	390	398	392	399				403	410
	25	392	391	394	392	393				399	403
	25	333	329	306	278	255				275	255

TABLE VII. Concentration of (4) in the Brain of Rats at Various Times After Administration of (5)

Time	Concentration ± SE
15 min	0.84 ± 0.36 μg/g
1 hr	2.34 ± 0.82 μg/g
2 hr	1.95 ± 0 μg/g

TABLE VIII. Anti-viral Activity of Ribavirin CDS's in a Punta Toro Mice Model[37]

Compound	Dosage (mg/kg/day)	Infected, Treated Surv/ Total	Infected, Treated MST[a] (days)
(26)	34.4	0/10	9.0*
	17.2	1/10	7.2
	8.6	2/10	8.4
	4.3	0/9	7.8
DMSO	-	1/18	7.8
Untreated	-	0/10	7.8
Normals	-	-	-
(5)	175	4/10*	8.3
	87.5	0/10	7.6
	43.8	2/9	8.9*
	21.9	1/10	7.9
DMSO	-	1/10	7.9
Untreated	-	1/18	7.8
Normals	-	-	-
(24)	28.1	0/10	8.3
	14.01	0/10	9.0*
	7.0	0/9	9.1*
	3.5	0/9	9.1*
DMSO	-	1/18	7.8
Untreated	-	0/10	9.0
Normals	-	-	-
(25)	25	0/9	8.4
	12.5	0/10	8.0
	6.25	0/10	8.5
	3.13	1/10	7.7
DMSO	-	1/18	7.8
Untreated	-	0/10	9.0
Normals	-	-	-

[a]Mean survival time of mice dying on or before day 21.

*$P < 0.05$